PROGRESS IN PHOTOBIOLOGY

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PROGRESS
in
PHOTOBIOLOGY

PROCEEDINGS OF THE THIRD INTERNATIONAL CONGRESS ON PHOTOBIOLOGY

The Finsen Memorial Congress

Edited by

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with a Preface by

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President of the Comité International de Photobiologie

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PREFACE

The Third International Congress of Photobiology was a special occasion for photobiologists all over the world, because it celebrated the 100th birthday of Niels Finsen. It was called The Finsen Memorial Congress.

The Comité International de Photobiologie was happy to receive an invitation for this congress from the Danish delegation at the Turin conference. It provided an opportunity to honor the memory of the outstanding man in the photobiology field, Niels Finsen, who did so much to lay a foundation for the development of not only photobiology, but also radiation biology in general.

The opening session was devoted to honor Finsen's memory with two lectures, one on his basic contribution to photobiology and the other on his contribution to the medical field. Also on the occasion of the congress The Dansk Esso Fund installed a memorial plaque in a building occupied by Niels Finsen for some time during his career in Copenhagen.

The congress was concluded with the presentation of the Finsen Medal to Dr. P. B. Rottier at The Finsen Institute where the Institute was host to the International Congress.

Besides the two lectures given at the opening of the congress, four lectures were presented by guest speakers: 1. Plant cell response to visible light — excluding photosynthesis by I. Virgin of Stockholm; 2. Light induced phosphorylation by D. I. Arnon of Berkeley, Calif.; 3. The effects of long visible and near infrared radiation by Hans Mohr of Tübingen; and 4. Biologische Uhren by Jürgen Aschoff of Heidelberg. Seven symposia were given: 1. Strahlungsklima und seine Messung — Chairman, R. Schulze of Hamburg; 2. Biological action spectra — Chairman, K. V. Thimann of Cambridge, Mass.; 3. Photoreceptors in aquatic organisms — Chairman, N. Millott of London; 4. Lupus vulgaris — Chairman, P. V. Marcussen of Copenhagen; 5. Initial mechanisms involved in radiation effects — Chairman, R. Latarjet of Paris; 6. Photoreactivation — Chairman, A. V. Giese of Stanford, Calif.; and 7. Phototherapy — Chairman, Jean Meyer of Paris. In addition to the 60 symposia lectures, 90 personal communications were given, abstracts of which are included in this volume.

The meeting discussed many questions on basic photobiology as well as applied photobiology with special emphasis on the development of photobiology during the past three years. Twenty-six nations represented at this congress indicates the increasing interest in this field and the international character of the meeting.

The congress itself was capably planned and managed by the President, Dr. B. Chr. Christensen, with the assistance of a competent staff. The result was a well-organized proceedings, most of which are being reproduced in this volume. Special credit should go to the Danish scientists and government as well as to the many contributors who made this congress possible. Mention should be made of the generous hospitality at several receptions by different organizations in Copenhagen.

At the close of my term of office, I would like to express appreciation to the Secre-

tary-General, Prof. Dr. W. Burckhardt, whose untiring efforts and willingness have done so much for the organization. I also would like to thank the Executive Committee who so graciously cooperated in preparing for this congress.

I would also like to take this opportunity to welcome Dr. Raymond Latarjet as the incoming President of the Comité International de Photobiologie.

ALEXANDER HOLLAENDER

CONTENTS

Plenary Sessions

Symposium 1

Strahlungsklima und seine Messung

Symposium 2

Biological Action Spectra

Symposium 3

Photoreceptors in Aquatic Organisms

Symposium 4

Lupus Vulgaris

Symposium 5

Initial Mechanisms involved in Radiation Effects

Symposium 6

Photoreactivation

Symposium 7

Phototherapy

Session 1

Plant Photobiology

Session 2

Animal Photobiology

Session 3

Photosynthesis

Session 4

The Reaction of the Skin to Radiation

Session 5

Photobiochemistry

Session 6

Photobiochemistry (especially Pigments) and Microbiology

Session 7

Photochemistry

PLENARY SESSIONS

OPENING OF THE THIRD INTERNATIONAL CONGRESS ON PHOTOBIOLOGY

THE FINSEN MEMORIAL CONGRESS

B. CHR. CHRISTENSEN

Isotope Laboratory, The Finsen Institute, Copenhagen (Denmark)

Your excellency professor Niels Bohr, dear members of the Finsen family, distinguished guests, fellow members of the Congress.

It is a great honour to us in Denmark to welcome The Third International Congress on Photobiology here this year. It is especially so because The International Committee on Photobiology decided to commemorate the 100th anniversary of the birth of the eminent Danish physician and scientist Niels R. Finsen by naming this congress The Finsen Memorial Congress.

To His Majesty King Frederik IX of Denmark, the Patron of our Congress, who by his patronage supported us in our work we owe our most wholehearted thanks. His Majesty regrets that he was unable to attend this opening because of a visit to Greenland. The Congress has sent a message of thanks to His Majesty.

We are very grateful to Mrs. Finsen who joined our honorary committee and by doing so assisted us so much in our efforts to make this Congress a dignified memorial to her husband Niels R. Finsen. We are very sorry that Mrs. Finsen cannot be with us to-day because of a sudden slight illness. We ask her daughter and son to convey to her our very best regards and most sincere wishes for a rapid recovery.

We want to thank our honorary president, his Excellency professor Niels Bohr, and the members of the honorary committee, the Danish Minister of Education, the honorable Jørgen Jørgensen, and the Director General of the Danish National Health Service, dr. Johs. Frandsen, for their assistance during the arrangement of the Congress.

As a whole the organization of this Congress has really been a wonderful experience since the name of Niels R. Finsen has made so many Governmental Institutions, Organizations, Funds and private persons assist us in our efforts. I therefore want to extend to all supporters our most sincere thanks.

This is the second time Copenhagen has the honour of being the host to a photo- or light congress as in 1932 the Second International Congress on Light Research was held here. Much has happened to science since then. We live in the era of specialization and under the pressure of the flood of scientific papers. Every minute, night and day, year by year, about 2000 pages are printed. If one of us wanted to study the publications relevant to his own branch of speciality and if he used all his time he would over a year miss more than half a million printed pages. Science really is in a state of fission, fission in the sense that each new discovery opens at least two new avenues for research. Thus our scientists are forced to know more and more about less and less. No person and no group is able to make a complete synthesis of all the

developments even in his own field. I do not know how to stop or alter this development — perhaps it cannot be stopped — but it is my sincere hope that by some process of fusion we may bring together all areas of science to find a simple solution to our complex problems.

Throughout these days we have here physicists, chemists, biochemists, biologists, botanists, plant physiologists, plant pathologists, physicians and many others gathered, to discuss problems of mutual interest. I consider a Congress like this, in which people from several branches of natural science meet to discuss a single topic — in this case light — as a step along the right track.

The great German philosopher Kant said that there were two outstanding wonders of God's creation. He said these were the light of the stars of the heaven above and the mind of man within. Niels R. Finsen's work was the result of a combination of these two. May we at this Congress witness a creative meeting of the light and the members of the Congress. With these words I declare The Third International Congress on Photobiology — The Finsen Memorial Congress — open.

Mesdames, messieurs

Au nom du comité organisateur danois j'adresse la plus cordiale bienvenue à tous au troisième congrès international de photobiologie — le congrès commémoratif de Finsen — exprimant la certitude que pendant les jours à venir nous assisterons à une rencontre inspiratrice entre la lumière et les membres du congrès.

Sehr verehrte Gäste und Kongressmitglieder

Im Namen des dänischen Organisationskomitees heisse ich Sie herzlich willkommen zum dritten internationalen Kongress für Photobiologie, — dem Gedächtnis von Finsen gewidmet. Ich bin überzeugt dass wir in den kommenden Tagen ein anregendes Zusammentreffen des Lichts und der Kongressmitglieder erleben werden.

FINSEN AND BASIC RESEARCH

ALEXANDER HOLLAENDER

Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn. (U.S.A.)*

We do not often have occasion to recognize pioneers in the radiation biology field. I consider myself highly honored to have been given the opportunity to call attention to the influence of one of the most important pioneers in the development of this interesting science. I am of course referring to the man whose memory we honor today — Niels Finsen.

When I first entered the field of radiation biology, coming from physical chemistry, more than thirty years ago, I made a survey of the material that had been published on the effects of light on living cells. It became immediately obvious to me that some of the most important fundamental work in this field had been accomplished by Niels Finsen and his coworkers.

I wrote to the Finsen Institute for information on Niels Finsen's work and received by return mail a set of the communications of the Finsen Institute**. A survey of these gave me a picture of the accomplishments of the Finsen group. I looked at these with admiration. Those of us who approached photobiology from the physical, chemical, and biological backgrounds look up to Niels Finsen as a most unusual medical investigator, who, in the early days, stood ready not only to use the tools developed empirically but who tried to understand the principles governing their action. He was interested in attempting to unravel the mechanism of the effects of light. His careful study of the comparative effects of sunlight and the effect of artificial radiation (especially the light from the carbon arc) is most impressive.

In his introduction to the communications of the Finsen Institute, Finsen divides the program of his new Institute into two sections. The first and most important purpose was to be the investigation of light and its effects on biological, chemical and physical systems. Only in this way would it be possible to provide a healthy and reliable basis for the second purpose of the Institute — the use of light in medicine. The first purpose requires investigations of a physical, chemical, and biological nature. The second requires practical medical investigation (research with different diseases). My discussion will deal mainly with the first part of the program outlined by Finsen. The next speaker will cover the second one.

If we survey the status of photobiology in the world before 1890, we find a general feeling that the region of the spectrum in the ultraviolet and visible was very important. However, this general attitude was expressed in only a very few articles. There

* Operated by Union Carbide Corporation for the U.S. Atomic Energy Commission.

**. Mitteilungen aus Finsens Medicinske Lysinstitut in Kopenhagen. Verlag von Gustav Fischer in Jena 1901–1906 inclusive.

is a very nice study on effects of light on bacteria by Ward and other studies on the effect of light on skin and general well-being of individuals, but no systematic quantitative study was conducted. This changed rapidly when Finsen entered this field.

In the early 90's Finsen presented a number of communications on the applications of his investigations to therapy, but they provided only a very general description of the improvements he had made on the equipment for the treatment of lupus vulgaris and other diseases. It was not obvious to the physicians or to the other scientists who read them that Finsen had developed, in the course of his background work, a number of very important basic physical and biological investigations on which these applications were based. Finsen had already described many of his pieces of equipment, and they had been used extensively in many countries outside Denmark, but he had not given his reasons for using lenses of glass or quartz, for preferring artificial light, for considering quartz so important for artificial light and less so with sunlight, for using distilled water instead of well water, for the advantage of using filters, and for using a lens system combined with filters filled with water dyed blue. However, all these things were based on his sound understanding and on definite quantitative investigations that he himself had developed, so that he had a background for his recommendations. Many people probably thought too that so much groundwork was really not necessary, and that they should simply use sunlight and forget all about the detailed directions he had to give. The basis of his method consisted of: *(1)* the use of bactericidal radiation, *(2)* the ability of light to produce vesication, and *(3)* a good understanding of how deep this radiation could penetrate.

It was obvious to him that for practical use of light it must be concentrated and, at the same time, the heat-producing radiation (infrared) had to be removed to avoid damaging the tissues. He stated his questions in the following way: which part of the spectrum has the most bactericidal effect? which part of the spectrum is connected with the damaging effect of radiation to the skin? what are the best methods to remove the heat radiation without interfering with the bactericidal and inflammatory effects of radiation? how deeply can the radiation penetrate into the tissue? Other questions that he asked himself were: what could one do to develop an artificial light source that was rich in radiation with the desirable properties and low in heat radiation?, what radiation from the electric arc is most desirable, that coming directly from the crater in the carbon electrode or that coming from the arc itself? and, finally, what is the difference between sunlight and radiation from an electric arc, and what importance has this in the design of an apparatus for the treatment of diseases? And a simpler question — what would be the best method of concentrating the light in such a fashion that the good properties are maintained while the heat radiation is removed? I believe that Finsen was the first one who clearly stated the problems with the practical use of light and definitely saw immediately the *physical basis* for some of the problems he encountered.

In an effort to study the bactericidal effect of radiation, Finsen worked with a *Bacillus prodigiosus* culture, a common water organism that we now call *Serratia marcescens*. In all studies the light was from a carbon arc. He spread the bacteria on the surface of agar plates and exposed them to the light, which had gone through a water filter. He covered the bacteria with a quartz plate, with a common window glass plate, and with a sheet of plate glass. He exposed the plates for 5 min. On the next day, all three plates showed some reduction of growth. The most striking

reduction was observed on the plate that received the light through quartz. Forty-eight hours later it became obvious that the most marked effect was produced by the radiation that had gone through a quartz plate, and almost no effect was observed on the plates exposed to light through window glass or through plate glass. Finsen reached several interesting conclusions on the basis of such experiments, one of which was that all light that penetrates glass (that is, window glass and plate glass) will delay division but that the killing effect of the light is greatest when it has passed only through quartz.

Some of these observations were overlooked for 40 years, when they were dug out again in a systematic study of the effects of light. Dr. Valdemar Bie and Dr. Sophus Bang, his close coworkers who continued these studies, later on observed that a 35-min exposure, even through a glass apparatus, could kill the bacteria. In contrast to this, Finsen could obtain the killing effect of the radiation in a few seconds if he used quartz plate. This result was obtained with light from a carbon arc which of course has a considerable amount of radiation at wave lengths below 3000 Å. However, when he used sunlight available in Copenhagen, he found very little difference in the effectiveness of the light whether he used quartz or glass, *i.e.*, the proportion of effectiveness of quartz to glass was about 5:4. On the basis of these experiments, Finsen introduced immediately the use of quartz plates or lenses in treatment with artificial light. Later also Bie and Bang established that light of wave lengths shorter than 3000 Å, especially about 2500 Å, was most effective.

Dr. Finsen made many experiments to determine the light path through the light-gathering power of lenses of different designs; he went very carefully into these questions, which can only be touched on here. He found, for instance, that a quartz lens could be placed very close to the arc, but a difficulty arose then in that the carbon particles soiled the lens very rapidly. In spite of the quartz lenses being considerably more expensive than the glass lenses, he definitely stated that the advantages of quartz lenses for use with the carbon arc far outweighed the extra expense.

Another point he wanted to straighten out was which part of the spectrum had an inflammatory effect on the skin. This he did very simply by comparing the effects of ultraviolet and visible light. Of course he immediately found that the light that was most bactericidal was also the same light that produced vesication of the skin. On the basis of this he concluded that the best lens for production of inflammation with artificial light (*i.e.*, the carbon arc) would be a quartz lens; with sunlight however there was little difference in the effectiveness of quartz and glass lenses in transmitting inflammatory radiation. Glass lenses were preferable, therefore, because they were inexpensive and could be obtained in large enough sizes, which was important in connection with the use of sunlight.

We then come to his analysis of what should be done about separating infrared radiation, which would produce a thermal burn, from the useful bactericidal and inflammatory effects of radiation. Stated categorically, to remove all thermal effects of light would be a physical impossibility since all radiation has the property of producing heat; only the *degree* of production of heat in human skin is different for different radiations. By checking carefully he found that patients could not stand more than 45–55°. Long exposure to temperatures higher than that had a very detrimental thermal effect. In his systematic manner he asked himself, in regard to the absorption of radiation by water, what percentage of the infrared is absorbed?

what is the absorption of ultraviolet or bacteriocidal radiation? what importance has the depth of the water layer? what importance has the temperature of the water? what are the absorption capabilities of different solutions that could be used to remove the biggest part of the heat radiation and the least of the ultraviolet radiation? He measured the temperature without water filter and found it to be 325° in front of his arc; then he filled the container with water, and the temperature went down to 111°. In his very precise manner he gives the spread of measurements, *i.e.*, the average of six measurements that had varied between 89 and 120°. The layer of water he used for these studies was 13 cm deep. He ran into all kinds of technical difficulties; anybody who has worked with a carbon arc can readily understand this. He especially had difficulty with the wandering of the arc. The arc would move back and forth at the electrodes and would not give a very steady, uniform light; however, for his purpose, this was not too serious a problem. He concluded that, since pure water does not absorb visible or ultraviolet light, the absorption was entirely in the infrared region. Then he switched to a different type of filter. The first filter he used was iodine in carbon disulfide, which is an excellent filter that permits infrared radiation to penetrate and absorbs all visible and ultraviolet radiation. When he put this filter behind the water filter he showed that the iodine filter was not heated by radiation, indicating that for all practical purposes the water filter had absorbed most of the infrared radiation. He then checked on the absorption quality of pure water for bactericidal radiation and found, of course, that very little if any of the ultraviolet radiation was absorbed. He found water filters even better than the empty filter vessel, because the ultraviolet radiation would penetrate better through the water filter than through the empty vessel. Of course, he explained that this was caused by reflection on the different surfaces of the filters. It was important to Finsen to determine a proper depth of the water layer: should it be a thin layer or should it be very deep? A deep layer would of course be technically very difficult to handle, whereas with a thin layer one had to be sure that it absorbed all the infrared radiation. He gives a very interesting table and shows the variation of the absorption of the water for infrared radiation in layers from 9–26 cm depth; the temperature at the focus varied from 325° with no water filter to 170° with 26-cm depth. However, the water filter caused little variation after it reached a depth of about 3½ cm, showing that there is really no advantage of more than 4-cm depth. He concluded, therefore, that 5-cm depth was more than adequate for his work with sunlight. For use with artificial light, the lenses were small and a deep layer of water could be used without too much difficulty, so he increased the water layer up to 35 cm. Apparently in his experiments, it made little difference in the results whether the water was kept at 5° or 73°. Later on, however, Finsen changed his mind in regard to this because he soon found that the water filter itself would become a secondary source of radiation and, if the water became too hot, it might produce heat effects that were undesirable. On the basis of Dr. Absalom Larsen's work in his laboratory, he concluded that it was absolutely essential to have freshly distilled water in all his equipment. It was important because not only would the contamination form a precipitate and light would be scattered, but also freshly distilled water would have all air removed so that no bubbles formed in the filter.

He very quickly found that adding a small amount of methylene blue to the water made it a much better absorber of infrared radiation. In a systematic study to find

the best way to remove the infrared radiation without interfering with the effective ultraviolet radiation, he examined methylene blue (an alkaline dye) and an ammonia solution of copper sulfate. The latter was by far the better absorber of infrared radiation, but it attacked the metal surface of the water container. Methylene blue was somewhat less effective but was much easier to handle. He carefully studied the effectiveness of these two solutions used in conjunction with each other. Using a combined filter he found that the copper-sulfate solution permitted more ultraviolet radiation to pass than the methylene blue. He solved the difficulties of interaction between the solution and the metal surfaces by covering the metal surfaces with lacquer. But even the copper-sulfate solution absorbed too much of the ultraviolet radiation, and since with the use of artificial sources this was an important point, he avoided this solution or used an extremely dilute solution.

The next question was what could be done to help the ultraviolet penetrate the skin; for this he designed a special method. He pressed the lens and filter directly to the skin, which forced the blood away, thus decreasing the absorption and improving the ultraviolet penetration. To prevent the apparatus from becoming a source of irritating infrared radiation, he designed very simple pieces of equipment, such as a quartz container (a better heat conductor than glass) that could be pressed against the skin. Also, he saw to it that water circulated through this lens filter system. The pressure apparatus would remove the blood and the circulating water would cool the skin. Practically speaking, this was extremely important since the cooling effect of the circulating water had a beneficial effect in alleviating the sensation of burning produced by the ultraviolet radiation. This changed the whole nature of the practical use of this radiation. All pressure vessels, even for sunlight, were subsequently made of quartz.

Finsen's first equipment for concentrating sunlight was awkward and very difficult to handle. The vessel would contain as much as 10 liters of water with a massive glass lens on each end. It must be remembered that at that time it was very difficult to get large lenses. He eventually devised one of his own by using a watch glass, which he filled with water and held against a glass plate. This system worked fairly well, but at that time watch glasses were imperfect and he never really achieved the optimum condition, especially for sunlight. Of course, when he changed from sunlight to the carbon arc, which has so much greater intensity in the ultraviolet and is a fixed source, much simpler design was possible. This was especially true when he obtained quartz lenses and used the long water column, which served so well as a cooling unit for the radiation. He encountered many difficulties, since the optical industry as we now have it did not exist at that time. He had to design his own lenses and equipment - - and he did an excellent job, as you can see when you visit the Finsen Museum here where many of his pieces of equipment are on display. He had to have a good understanding of optics to design the equipment so that he could get a uniform spot of light on the skin. In using the light beam, he had to take precautions that the area was uniformly irradiated and finally decided on long-focus lenses, which simplified the problem considerably. With modern equipment, this is a comparatively easy problem, but at that time it was difficult and awkward. Also, he constantly had to switch between sunlight, in which the light comes in fairly parallel, and an artificial source — the carbon arc — which is a point source and requires a differently designed apparatus to be effective.

His most interesting design of apparatus was for the electric arc, which can very well serve as a model for modern design of equipment. In all these designs and plans for his work, he had to consider the economics as well as the effective scientific design, a design that would enable him to irradiate as great an area as possible at as great an intensity as possible. Since he often had to expose many patients at one time, the apparatus was designed accordingly. If you visit the Museum you will see how intelligently he went about this.

Then he had to decide in which cases to use sunlight and in which ones to use artificial light, especially since sunlight contained much infrared radiation and a very small amount of far ultraviolet. In the carbon arc, the reverse was true. Also, the ultraviolet available from sunlight in northern latitudes was not very abundant. He concluded that the electric light was much better for practical treatment. He designed a 20–25 cm diameter lens for use with sunlight and used a much smaller lens with the carbon arc. He used a carbon arc extensively at about 70 A, which is still moderate in infrared production that could be handled by the type of equipment he had. Time and again he discussed the effectiveness of the light in killing bacteria. With sunlight, several minutes would pass before an effect could be recognized; but with artificial light from a 70-A carbon arc an effect required only a few seconds. In spite of all the theoretical differences and advantages of artificial light, there was some tendency at the Finsen Institute to give preference to sunlight, because its mild radiation effect and lack of short ultraviolet had a generally better effect than the carbon arc. The preference for sunlight treatment may also have been influenced by the fact that patients were treated in the summertime, when they were out in the fresh air and probably had a general feeling of well-being. Also, in the summertime the lupus infection would tend to be much milder or would disappear entirely. With other diseases of the skin, however, this was not so, and here the concentrated radiation from a carbon arc was much more effective.

Of course we always ought to remember, and my own tendency is to forget this, that there is very little sunlight in cities of the North except during the summer months, which probably was one factor that encouraged Finsen to develop the carbon arc. The area where I come from has bright sunlight with a reasonable amount of ultraviolet radiation even during the wintertime, except for a period of say 8 weeks.

Finsen was very much interested in the scattering and attenuation of solar radiation as it traverses the atmosphere. In particular, he gave many discussions of the red color of the setting sun as well as the changes in infrared and ultraviolet composition of sunlight reaching the earth's surface as a function of the sun's elevation. In addition his coworkers, for example, Larsen the physicist and his group, made excellent intensity measurements of total sunlight. They found, for instance, that in summer he amount of ultraviolet would have the effect of a factor of 119 at noon, whereas at 7:00 P.M. it would have only 5, in spite of the fact that on the 21st of June there are almost 20 h of daylight. They found that the sunlight at noon on the 21st of December contained as much ultraviolet radiation as at 7:00 P. M. on the 21st of June.

Then finally, as a good scientist, Dr. Finsen worried about the shortcomings of his methods and wanted to know what could be done to improve his sources. Here he emphasized again that the radiation should be as rich as possible in the ultraviolet and as poor as possible in the infrared. In regard to sunlight, he pointed out that its location on the surface of the earth and the times when sunlight is used are important

factors. I hardly need to point out the great influence this had on the development of outdoor sanitoria in places like Davos, where the atmosphere is clear and the location fairly far south and where there is a minimum interference with the sunlight. Modern developments in medicine, *e.g.*, the antibiotics, have made many of these institutions obsolete. He pointed out that Switzerland was probably a good place to develop these sunlight treatments and Greece was even better. He had many suggestions for improvement of the apparatus to handle irradiations.

I think the generosity with which Finsen as an investigator treated his coworkers should be pointed out. He did not hesitate to mention each one who had worked out something and he did this in a very systematic manner. The names of Larsen, Bang, Bie, Busck, Jensen, Schmidt-Nielsen, and many others were connected with the accomplishments of Finsen.

If we consider that Finsen started out long before X rays were discovered, that he saw the importance of physical, chemical, and basic biological studies conducted so that quantitative data were obtained that could help to unravel the mechanism of the phenomena, we feel more what a tragedy it was to lose him so early in his lifetime and can only humbly give our respects on his 100th birthday.

FINSEN AND MEDICAL RESEARCH

POUL V. MARCUSSEN

The Finsen Institute, Copenhagen (Denmark)

In medical research Niels Finsen is known primarily for the studies and experiments which led to his treatment of *lupus vulgaris*. When on November 20th, 1895 he started treating his first patient with concentrated and filtered light rays from a powerful arc lamp, he was creating a new therapeutic method for an hitherto incurable disease. Niels Finsen's first patient was typical of the *lupus* patients of those times. He had undergone 37 surgical operations which nevertheless had failed to arrest the progression of the disease. After having had light therapy for 4 months he was practically cured.

To posterity it must seem to have been by chance or by a stroke of genius that Niels Finsen chose *lupus vulgaris* to illustrate the value of his therapeutic principle. At that time he was only 34 years of age and he was cultivating his anatomical and surgical interests with great talent. He was already known as an able experimenter, but his previous biographers[9] have been of the opinion that he was but slowly accepted as a scientist. Repeated perusal of Niels Finsen's first publications[1-7] however, reveals that he must have belonged to the rare group of scientists who form their scientific philosophy at an early age and go on elaborating it by observations and experiments throughout life. We know that as early as the age of 26 he formed an opinion of the biological effect of light which he considered to be predominantly beneficial. His observations of nature and the results of some preliminary experiments upon himself led to the development of three valuable phototherapeutic methods from this basic idea. In 1893, at the age of 31, he published his treatment of small-pox, excluding the undesirable rays by red filters[1]. This was a purely logical deduction based on known facts, but its value was soon confirmed by others. After having studied the detrimental effects of light for one or two years[2], Finsen reverted to its presumed beneficial effect, finding support in studies of the seasonal variations of the haemoglobin level[3] in 1894. The following year he published comprehensive investigations into the effect of various rays upon the movements of lower animals[4]. By 1896 he had adopted the views of modern bacteriology, trying to explain part of the beneficial effect of light by a bactericidal effect[5]. He was the first to adopt the physical laws on quantity, quality, absorption and penetration in phototherapy, and predicted that it ought to be possible to construct an apparatus able to kill bacteria in the superficial layers of the tissue within a reasonable time. In a brief appendix to his paper he reported the results of treating 11 patients suffering from *lupus*. Only one of them, however, had been followed up for any length of time. The choice of *lupus* as an experimental subject is quite astounding, but without doubt this was just another manifestation of Niels Finsen's talent for logical deduction. Finsen

himself states that he chose *lupus* because it was a superficial, local, bacterial disease, because the *tubercle* bacillus was known to be suspectible to light, and because for this disease "a new and better method of treatment was needed". Because such a deduction can anticipate discoveries which would not otherwise be made for decades or even centuries and thus alleviate much human suffering, I have investigated other theories on this subject, particularly the influence of earlier surgical literature and the special consideration of the first patient, who was a friend of the director of the laboratory of electrophysics where Niels Finsen performed his first experiments. On another occasion I hope to be able to submit the results of these investigations. They appear to prove that any other influence may be ruled out and that the choice of *lupus vulgaris* was exclusively a result of scientific thinking.

That Niels Finsen had to fight for his ideas is no doubt correct. But his victory was quicker and more convincing than in analogous cases. As early as August 1896 he was able to open his first, though primitive, Research and Therapeutic Institute. This is another proof that his was an elaborate and well-founded theory which could convince a group of critical experts although at this juncture the treatment of the first patient had not even been completed. The treatment of *lupus* gained great practical importance. After the method had been submitted to the 13th International Congress of Medicine[6] in Paris in 1900, it was adopted in practically all European countries, in Egypt, in Japan, and in the U.S.A. It prevailed as the sole treatment until vitamin D_2 therapy was introduced by Charpy and Fanielle. In the latter method too Finsen therapy was used as an adjuvant, and even after the advent of chemotherapy in tuberculous diseases, Finsen therapy will still be useful in cases where bacterial resistance develops. Not all the details of Niels Finsen's ideas have been fully confirmed. This applies particularly to the effect of light upon bacteria in the tissues which has proved to be limited by physical conditions. However, his basic view that the effect of light on *lupus vulgaris* is a photochemical process was confirmed in 1958 by the interesting findings of Van der Lugt and Rottier, both of whom are present at this Congress. Niels Finsen's medical recommendations in relation to artificial general light-baths have influenced a large number of scientists. Moreover, the concept of sunlight as a source of health has completely altered the attitude of the population in the northern lowlands.

Let me finally revert to *lupus vulgaris*. Niels Finsen centralized and organized its treatment in a way which to this day has permitted study of the disease on a nationwide basis as well as trial of other therapeutic methods. Now, in 1960, one hundred years have passed since Niels Finsen was born. It is also the first year in which no fresh case of *lupus* has been received for treatment in the Finsen Institute in Copenhagen, where only two resistant cases of this disease are now being treated. Altogether some 5000 *lupus* patients have been treated at the Institute and of these 4000 have been cured by Finsen's method alone. Thus, the eradication of *lupus vulgaris* from Denmark is primarily due to the work of Niels Finsen.

References p. 14

REFERENCES

[1] N. R. Finsen, Om Lysets Indvirkninger på Huden, *Hospitalstidende*, 36 (1893) 121.
cf.(a) *La Photothérapie*, Carré et Naud, Paris, 1899.
(b) *Über die Bedeutung der chemischen Strahlen des Lichtes für Medizin und Biologie*, F. C. W. Vogel, Leipzig, 1899.
(c) *Phototherapy*, Edward Arnold, London, 1901.
[2] N. R. Finsen, Om de kemiske Strålers skadelige Virkning på den dyriske Organisme, *Hospitalstidende*, 36 (1894) 1069.
[3] N. R. Finsen, Om periodiske årlige Svingninger i Blodets Haemoglominmaengde, *Hospitalstidende*, 37 (1894) 1209.
[4] N. R. Finsen, Lyset som Incitament, *Hospitalstidende*, 38 (1895) 205 (*cf.*[1] a, b, c.).
[5] N. R. Finsen, *Om Anvendelse i Medicinen af koncentrerede kemiske Lysstråler*, (Med en foreløbig Meddelelse om Methodens Anvendelse ved *Lupus vulgaris*), Gyldendal, København, 1896; *Mitteilungen aus Finsens Med. Lysinstitut, Kopenhagen*, F. C. W. Vogel, Leipzig, 1902, III (*cf.* ref.[1] a, b, c).
[6] N. R. Finsen, Traitement du *Lupus vulgaire* par les Rayons Chimiques, *XIIIe Cong. Intern. Méd., Paris, 2–9 août 1900*, Masson et Cie, Paris.
[7] N. R. Finsen, *Die Bekämpfung des Lupus vulgaris*, Fr Bagge, Kopenhagen, 1902, Fischer, Jena, 1903; *La lutte contre le Lupus vulgaire*, C. Naud, Paris, 1903.
[8] N. R. Finsen und H. Forchhammer, Resultate der Lichtbehandlung bei unseren ersten 800 Fällen von *Lupus vulgaris*, *Mitteilungen aus Finsens Med. Lysinstitut, Kopenhagen*, Fischer, Jena, 1904, V – VI, p. 1.
[9] Biographies: W. Thulstrup, Schønberg, København, 1910.
Hasselbalch, København, 1942.
Sv. Lomholt, Gyldendal, København, 1943.

PLANT CELL RESPONSE TO VISIBLE LIGHT EXCLUDING PHOTOSYNTHESIS

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The living organism has many times been compared with a factory. In the green plant factory, light energy from sun is the main fuel to drive the engines — the green chloroplasts — by means of which the plant is able to build up carbohydrates which later on can be converted into other chemicals.

The engines in a factory need maintenance in order to work properly and must be kept in order and be continuously adapted for the variously occurring needs and conditions. For this attention energy is required, distributed through several different departments in the factory. This is also true for the green plant. While photosynthesis and production of the plant body is the main energy-consuming process, the main operation of the factory as it were, a number of other less energy-consuming but nevertheless very important processes are grouped around it in order to make the conditions as favourable as possible for this main process to run. Many of these processes also need light energy to proceed, although the light in some instances is working more as the trigger mechanism than as the energy source for the reaction. In this paper, stress will primarily be laid upon some of these other light energy-consuming processes — mainly those which are brought about by light in the blue part of the visible spectrum.

There is a fundamental physical law which states that light cannot exert any effect on an irradiated system unless it is in some way absorbed. Since the plant cell is rich in pigments which can absorb different wavelengths from white sunlight, the natural light source for plants, we can expect that many reactions will be brought about by the light through its absorption by pigments. The various pigments are often connected with special chemical systems and we can also expect that — depending on the quality of the incident light — different light-dependent reactions will be initiated.

Upon irradiation with white light all reactions will proceed simultaneously but by using monochromatic light, or light from only limited parts of the spectrum, it may be possible to distinguish between different light-dependent reactions. In this way the action spectrum for the process in question can be established and from this action spectrum we may be able to deduce the pigment or pigment systems responsible for the reaction. The photoprocesses in higher plants seem to a great extent to be regulated by five photochemical reactions which are working more or less simultaneously although there is some interdependence. As photosynthesis is the main reaction all the others are to a certain extent dependent on the products of this process. There is also evidence which indicates some connection between several of the

References p. 26

reactions which earlier were considered to work independently of each other. We can here distinguish between *high-energy reactions* and *low-energy reactions*. The high-energy reactions proceed at light intensities above about 100 ft.-candles if we consider unfiltered sunlight, while the low-energy reactions require much lower intensities. The main high-energy process in the higher plants is of course photosynthesis which requires intensities between about 100 and 1000 ft.-candles to produce any appreciable amount of material. The compensation point is in most cases at intensities of about 100 ft.-candles and even if the photosynthetic process is working below this point there is no net production of material. Table I lists, as far as we know, the principal photochemical reactions, which should be taken into account in studies of green plants.

TABLE I

PRINCIPAL PHOTOCHEMICAL REACTIONS OF HIGHER PLANTS[18]

Photoprocess	*Reaction or response*	*Products*	*Photoreceptors*	*Action spectra peaks, mμ*
		Energy conversion		
Chlorophyll synthesis	Reduction of protochlorophyll	Chlorophyll *a* Chlorophyll *b*	Protochlorophyll	Blue 445 Red 640
Photosynthesis	Dissociation of H_2O into 2 H and $\frac{1}{2}O_2$ and reduction of CO_2,	Reductant H Phosphorylated compounds	Chlorophylls Carotenoids	Blue 435 Red 675
		Regulation of growth		
Blue reactions	1. Phototropism 2. Protoplasmic viscosity 3. Photoreactivation	Oxidized auxin, auxin system and/or other components of the cell	1. Carotenoid and/or flavin 2. Unknown 3. Pyridine nucleotide, riboflavin, etc	1. Near UV: 370 Blue: 445 & 475 2. Uncertain 3. Uncertain
Red, far-red reactions	1. Seed germination 2. Seedling and vegetative growth 3. Anthocyanin synthesis 4. Chloroplast responses 5. Heterotrophic growth 6. Photoperiodism 7. Chromosome response	Biochemistry completely unknown	Possibly tetrapyrrole	1-6. Induction by red: 660; reversal by far-red: 710 & 730 Far-red induced, red reversed, spectral details uncertain

The first process listed is the synthesis of chlorophyll *a* from its precursor protochlorophyll. It is well known that plants in darkness do not form any green pigments although small amounts of protochlorophyll are always present in all green plants which are kept in darkness. This substance is transformed rapidly into chlorophyll *a* by light[1] and we know that the light which is active in this process is that which is absorbed by the protochlorophyll. This means that the action spectrum for the process will be very like the absorption spectrum for this substance with two peaks – one

in blue and one in red. Even the formation of the precursor protochlorophyll seems to a certain extent to be dependent on a light reaction, both red and far-red light playing some role[2,3].

Secondly we have the main photoprocess of plants, photosynthesis. The chlorophylls are the principal photoreceptors for this reaction although energy absorbed by other pigments can be used after transference to chlorophyll *a*[4].

The next group of reactions is initiated by blue light. Although it has not yet been definitely proved it is thought that the photoreceptors in these reactions are located in the cytoplasm itself while in the reactions mentioned above, chlorophyll formation and photosynthesis, the receptors are located in the chloroplasts or the so-called proplastids, the small bodies which are later transformed into chloroplasts. Among reactions belonging to this group, we have first and foremost the phenomenon of phototropism. This is the visible result and the last step of a long series of reactions started by the absorption of blue light in some kind of yellow pigment the nature of which is not yet clear. Phototropism — the bending of shoots towards or away from a light source — must of course have a cellular origin. There are several responses of the single cell known which may be connected with this process. Variations in the consistency or viscosity of the cytoplasm and alterations in protoplasmic streaming under the influence of light have been rather well investigated and will be particularly dealt with in this survey. Blue light also has a reactivation effect on cells which have been irradiated by ultraviolet light. The action spectrum for this process indicates an absorption by pyridine nucleotides or riboflavin[5].

Finally we have a long series of reactions which are initiated by light in the long wave region of the visible spectrum. Because they intervene in so many important aspects of plant development, such reactions seem more and more to attract the attention of plant physiologists. The interesting feature of these phenomena is that

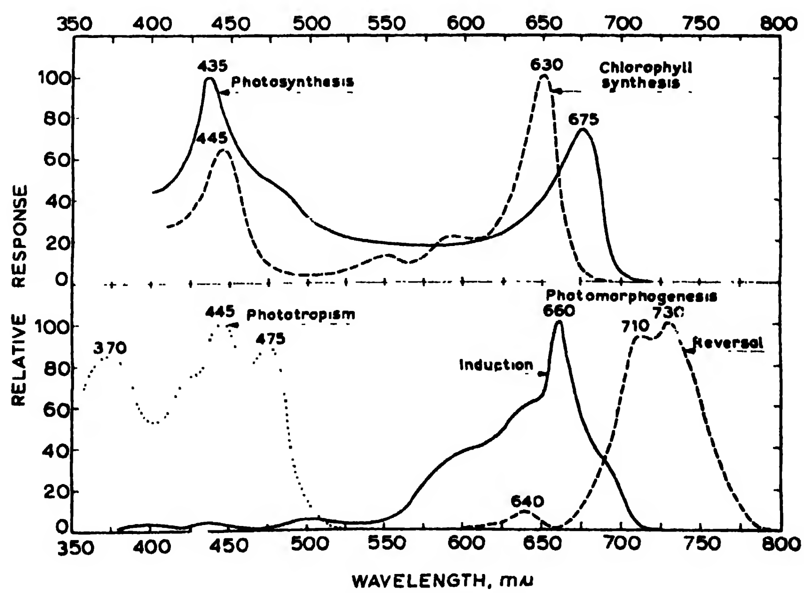

Fig. 1. Action spectra for the main photochemical reactions in the plant[18].

References p. 26

we have here a reversible system in that reactions initiated by red light can become nullified by a subsequent irradiation with far-red light. Among these reactions we have one in which far-red light seems to increase the frequency of X-ray-induced chromosomal aberrations. This effect is reversed by red light[7]. The action spectra for these main photoresponses are seen in Fig. 1. In Table II are shown some values for a few important responses for the range of light intensity from full sunlight down to darkness. From Table II it is clear that the cell of the living green plant responds to a tremendous range of light intensities and one can say that intensities from zero up to full sunlight are effective in moulding the shape of the plant.

TABLE II

RANGE OF LIGHT INTENSITIES FOR BIOLOGICAL RESPONSES (Withrow, 1959)[18]

Irradiance (log μW/cm²)	
5	Sunlight, noon, clear sky, June (10,000 ft.-c.) Photosynthesis saturates, wheat (2000 ft.-c.)
4	Sunlight, noon, overcast sky (100–1000 ft.-c.)
3	Photosynthesis, compensation point
2	Photoperiodic control of flowering
1	
0	End of twilight (0.3 ft.-c.)
−1	Limit of flower-bud induction (0.01–0.1 ft.-c.) Moonlight, full moon (0.02 ft.-c. maximum)
−2	Limit of cone or color vision Limit of detectable chlorophyll synthesis (red)
−3	Threshold of phototropism, Avena tip (blue)
−4	Threshold of bean hook response (red)
−5	Limit of rod vision, dark-adapted eye
−6	
−7	
−8	Threshold of photomorphogenesis (red) Avena first internode, Bean hypocotyl

It is probably a long time since man first observed that shoots and flowers in general bend towards the sun. We observe that our potted plants in the windows sooner or later orientate their leaves towards the light of the sky. This phenomenon of phototropism was first studied scientifically by Darwin, but the most penetrating studies were performed by Dutch plant physiologists at the beginning of this century. Since that time particular interest has been focused on the behaviour of that part of the grass seedling which first emerges from the ground, the so-called coleoptile which encloses the first leaves. One might say that hardly any part of the plant has been so thoroughly and carefully studied as this little pale cylindrical plant organ only 3–4 cm long.

A characteristic feature of the coleoptile is that it is extremely light sensitive and will bend towards, or away from, a light source depending on the amount of light energy administered. The phenomenon of phototropism is a characteristic feature for all growing shoots and sometimes root tips show a similar response. If they do, they usually bend away from the light source. The literature on this phenomenon is enormous and the researches reported include all kinds of irradiation experiments more or less ingeniously arranged. The phenomenon of phototropism is not restricted to higher plants since similar responses are found throughout the whole plant kingdom. The bending of the sporangiophores of certain lower fungi have also been extensively studied.

It quite early became clear that the bending was the result of a different elongation of the cells on the irradiated and non-irradiated sides of the organ. The question of whether the organ responds as a unit or whether the bending is the result of light–growth reactions of the single irradiated cells was not solved until around 1911, by the Danish plant physiologist Boysen-Jensen; Paal, Stark and later Went in 1928 definitely proved that the bending was caused by the presence of a chemical substance. The growth of the organ itself is thus due to a growth-promoting substance which is continuously formed in the very tip of the coleoptile from where it migrates downwards. During its passage to the basal region it affects the cells it passes. The effect of light on the coleoptile and on shoots in general, if we now restrict ourselves to the phenomenon of phototropism, thus consists of an influence on this stream of growth substance so that — in the case of bending towards the light source — more of the substance is moving along the dark side of the irradiated organ than along the irradiated side. The finding of this cause for cell growth in general gave rise to tremendous research activity in this field. How the stream of growth substance is affected has not yet been discovered, and this is a very real problem in plant physiology today.

What do we now know about the primary light action in this process? It would be a flagrant lie to say that we do not know very much since the literature on the subject is very comprehensive, but the truth is that in spite of this we still do not know for sure which substance absorbs the light. It was quickly recognized that this reaction takes place only in blue light. If a coleoptile or shoot is unilaterally irradiated by a beam of red light the growth of the coleoptile may be changed, but it does not bend. From this it may be concluded that there must be a yellow pigment in the plant which is responsible for the light absorption. Although a great many experiments have been performed in order to find what this pigment is, the question has not yet been solved[8]. The present position can be expressed thus. It was soon found that the action spectrum for the process is very like the absorption spectrum for some carotenoids. From this fact the conclusion was drawn that carotenoids are the photoreceptors of the process as such compounds are present in the organ. Later experiments have shown that even organs having little or no carotenoid content respond phototropically and that there thus must be another substance which absorbs the light. Such a substance we have in riboflavin which is present in all living cells and it is found that its absorption spectrum shows similarities to the action spectrum of phototropism. There are differences in the location of the peaks of the absorption spectra of the two substances *in vitro*, but we do not know for sure their locations *in vivo*. Probably light absorption by both substances is active in the process. The obtaining of a true action spectrum for the process is rendered difficult by the fact that probably at least two

References p. 26

reactions are proceeding simultaneously quite possibly with different absorption mechanisms.

The visible results of a light stimulus must have a cellular cause. This is of course a truism. Nevertheless, it is an important fact to bear in mind. Not until we have been able to track down the primary effect of light on the single cell can we understand the further mechanism underlying the visible reaction. The plant cell is unique in the respect that it responds to visible light in so many different ways. It is possible to show experimentally that irradiation changes the general metabolism through changes in enzyme activity, ion uptake and the content of growth promoting substances. It also affects the viscosity of the cytoplasm, the permeability of the plasma membranes, the streaming of the cytoplasm and many other factors.

When it comes to find out possible connections between changes of this kind and responses manifested in a visible change like that of phototropism, one has to return to the action spectrum for the process. From the experimental results hitherto gathered it is more or less evident that changes in the cytoplasm itself, like changes in permeability and viscosity and to a certain extent also protoplasmic streaming and chloroplast movements, are initiated by long wave ultraviolet light and the blue part of the visible spectrum with a limit at about 5000 Å. As changes of this kind take place very rapidly, sometimes within a few seconds or a few minutes of the commencement

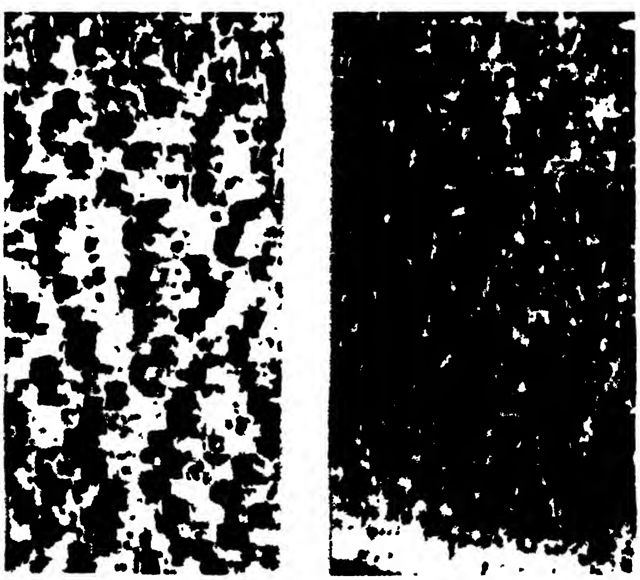

Fig. 2. Effect of centrifuging on leaf cells of *Helodea densa*. Left: centrifug[illegible] Right: not centrifuged. (Orig.)

of irradiation, it is clear that these effects always precede the redistribution and changes in hormone contents which are measurable after irradiation of shoots, coleoptiles and similar organs which show a visible phototropic response. But this does not, of course, necessarily mean that there is any further connection between the plasma changes and the distribution of growth substances. In my opinion, however, the different phenomena seem to be connected.

The effect of light on the viscosity of the protoplasm[10] can most easily be demonstrated by centrifuging irradiated cells. The contents of the cells which have different specific gravities from the cytoplasm will then move to the centrifugal or centripetal ends of the cells depending on whether their specific gravity is higher or lower than that of the cytoplasm itself. In cells containing chloroplasts, these particles move towards the centrifugal ends of the cells. By using centrifugal forces of the right value it is in this way possible to distinguish between cells with cytoplasm of different viscosity. Fig. 2 shows two parts of a leaf of *Helodea* — a common water weed — one part of which has been centrifuged so that a displacement of the chloroplasts has taken place. The magnification is about 100 times. Most of the chloroplasts have here assembled in aggregates at the centrifugal ends of the cells while the rest of the cells look rather empty. A centrifugal force of *ca.* 900 g was applied to achieve this displacement.

An approximate value for the protoplasmic viscosity can be obtained by determining the time in minutes which is required with a constant centrifugal force to get a displacement of the chloroplasts in about 90% of the cells. This value can be determined rather accurately due to the structural properties of the cytoplasm. The contents of the cells do not move gradually but when the chloroplasts start to move in a cell the further displacement will be finished within about 0.5 min — if they move at all. The fact that the cytoplasm only lines the inner walls of the cell wall also contributes to this kind of displacement. If centrifuging is carried out at short intervals

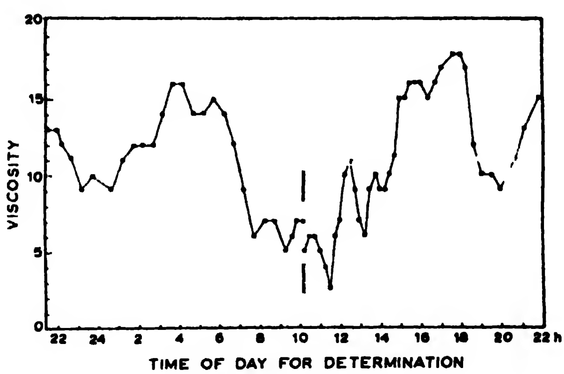

Fig. 3. Fluctuations in the protoplasmic viscosity during the course of night and day[10].

during the course of the day, it is found that the value for the centrifuging time fluctuates. The viscosity shows maximum values during the night and minimum during the day time (Fig. 3). The same kind of fluctuation can be measured in terrestrial plants[17] but as the consistency of the cytoplasm responds to changes in the water deficit of the plant, the conditions become more complicated.

Even under the influence of constant light, the protoplasmic viscosity shows fluctuations. It is thus never possible to get a constant value for the viscosity under the influence of light. The changes under the influence of natural daylight show a definite 24-h rhythm. This rhythm however, seems to disappear under the influence of constant light. In the latter case in contrast with the photoperiodic changes, the changes in

viscosity values show no signs of a regulation by any autonomous rhythm. The only way in which one can obtain a constant value for a longer period of time is to keep the cells in darkness for at least three consecutive days. After this period in darkness the value is fairly steady (Fig. 4), although when a constant value for the viscosity has been obtained in this way the cytoplasm is extremely sensitive to the influence of light (Figs. 5 and 6).

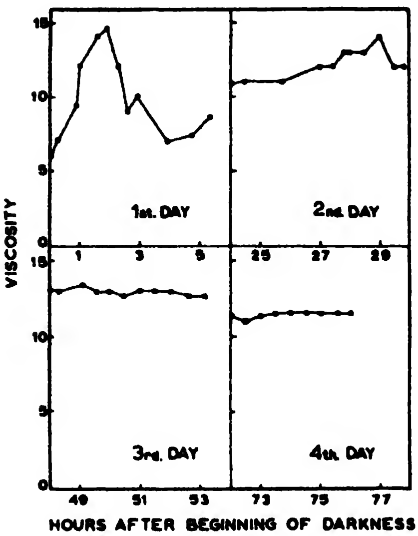

Fig. 4. Fluctuations in the protoplasmic viscosity during three days in darkness[10].

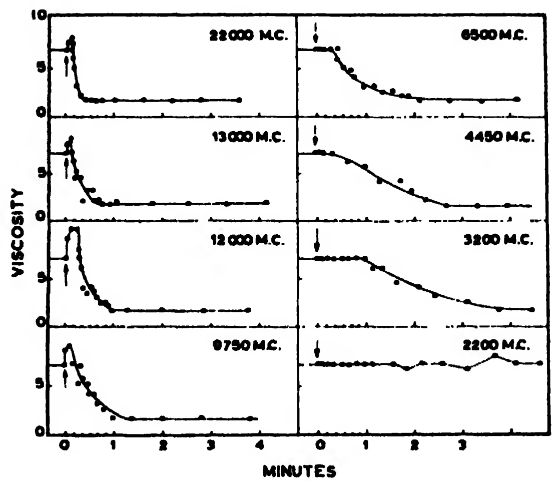

Fig. 5. Effect of continuous light on stabilize protoplasm[10].

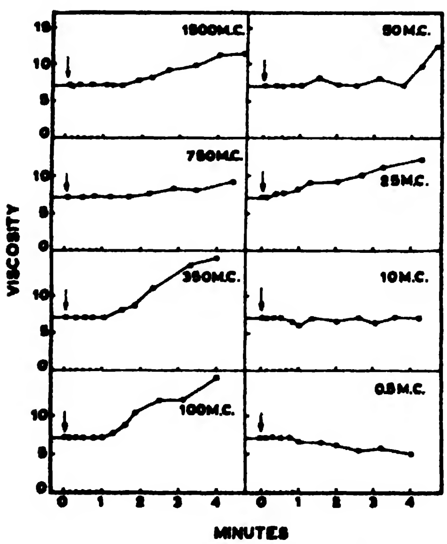

Fig. 6. As Fig. 5.

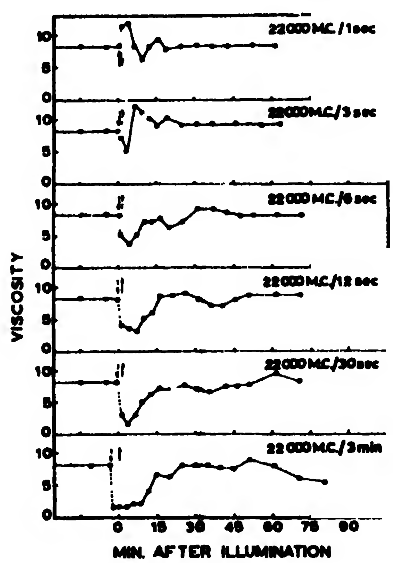

Fig. 7. Effect of short light impulses on stabilized protoplasm[10].

If we first look at the response during the first few minutes to different light intensities under constant irradiation it is found that the response depends on the intensity of the light. Except for a transient increase in the value for high light intensities which

might be due to experimental error, the most evident effect is a strong decrease of the value. A gradual decrease in the light intensity makes this decrease less evident and an intensity of about 2200 m-candles (about 200 ft.-candles) only causes fluctuations in the value around the initial value. Under the influence of still weaker light the first effect on the viscosity of the cytoplasm is an increase in the value. This increase takes place within an intensity range from about 1500 to about 10 m-candles. Still lower intensities of light again cause a decrease in the values similar to the first effect of the light impulse.

If, instead of using constant irradiation, the cells are irradiated with short impulses of light, characteristic fluctuations in the values are observed. One example from such an experiment is presented in Fig. 7. This series represents just one intensity of light — 22,000 m-candles — which has been given for varying short periods of time. The changes which are observed resemble very much the light-growth-reactions of a coleoptile which can be measured after a short light impulse, *i.e.* the different reactions are very similarly timed.

Fig. 8. Localized change in the viscosity of *Helodea* protoplasm, obtained by irradiation of a leaf through a slit in tin-foil, followed by centrifuging[9].

From experiments like these it has been possible to determine the threshold value for obtaining a response. The highest sensitivities hitherto measured on material cultivated during the winter is 6 m-candle-second for the threshold value for the minimum quantity of light which is needed to elicit a response. At the same time the absolute threshold value for obtaining a response after many hours of irradiation was as low as 0.001 m-candles. The values in absolute energy units have not yet been established. The threshold values are thus of the same order of magnitude as those found to apply to the phototropic phenomena. As far as light influences on viscosity and on permeability are concerned, it seems likely that the light effect is very local. This means that only the irradiated parts of the cells are affected and thus show a response to the light stimulus.

In Fig. 8 a leaf of *Helodea* is seen which has been irradiated through a slit in tin-foil and then centrifuged. Under the influence of the centrifugal force the chloroplasts — having a specific gravity higher than that of the surrounding cytoplasm — have moved to the centrifugal ends of the cells. As light in this case has caused a lowering

References p. 26

of the viscosity of the cytoplasm, the chloroplasts in the irradiated cells move faster than in the other cells. It is possible to discern a very sharp borderline between the irradiated and non-irradiated cells. It is also possible to distinguish between the irradiated and non-irradiated parts of a single cell (Fig. 9). As the cells respond so distinctly to the light it has been possible to use this method to obtain the action spectrum for the light action (Fig. 10). This figure shows a leaf of *Helodea* which has been irradiated by an energy-equalized spectrum with an energy gradient from the bottom to the top. After irradiation the leaf has been centrifuged. The result is an action spectrum produced directly on the leaf, formed by the cells in which the chloroplasts have moved due to the decrease in plasma viscosity. The picture is a combined photograph from ten single measurements. The spectrum obtained in this way is a typical blue-type spectrum similar to that of phototropism although in this spectrum it is not possible to find any definite peaks. It is thus not possible to make a detailed anal-

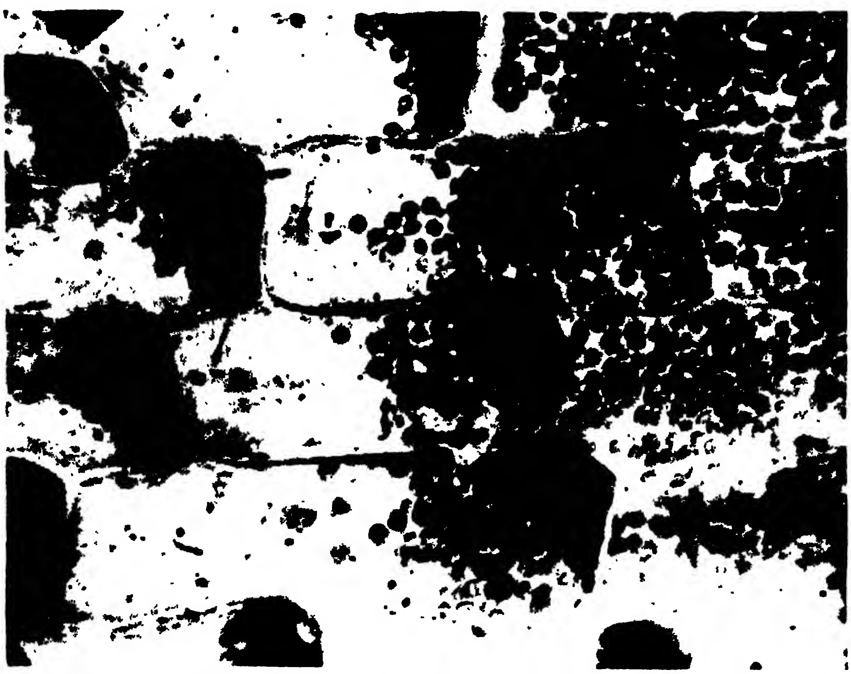

Fig. 9. The borderline between irradiated and non-irradiated area[10], *cf* Fig

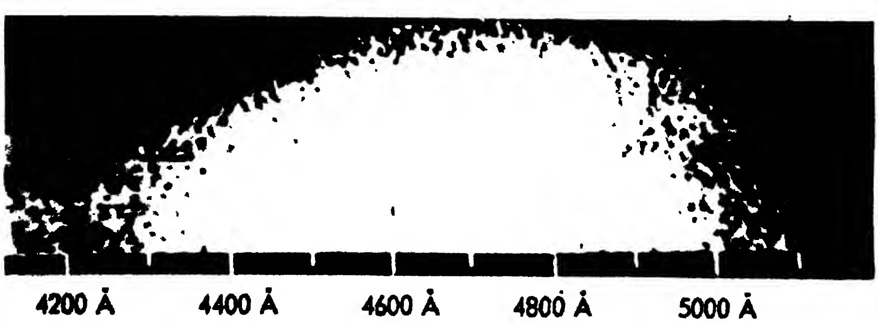

Fig. 10. Action spectrum for the light-induced change in protoplasmic viscosity. This action spectrum has been obtained by the projection of a light-spectrum with a vertical energy gradient directly on a leaf, followed by centrifuging[11].

ysis. Earlier measurements on single leaves had revealed that there are indications of two peaks in this action spectrum but the spread between the single determinations is so great that the results are not quite conclusive. However, everything points to a great similarity between the spectra.

The effect of light upon the viscosity of cytoplasm has been demonstrated in different species of green plants, aquatic as well as others, and there is reason to believe that it is a general phenomenon. One may therefore suppose that there exists a correlation between this phenomenon and phototropism. If blue light has a general influence on cells and such an influence has also been proved to exist in cells of coleoptiles - phototropic bending will be the result, in the case of a polar organ with a continuous flow, of growth hormones in one direction. It should be emphasized however, that we still do not know how a possible change in the plasma viscosity can affect the transport of hormones. Also, no effect of light on the transport rate of growth substance has hitherto been demonstrated. The cellular changes behind the visible phenomenon of phototropism must thus still be considered an unsolved problem.

Among other effects of light on the cytoplasm which probably belong to the same group we may consider that on the protoplasmic streaming. This effect would appear to be a very complicated one, for it seems that we are dealing here with two simultaneously occurring reactions, firstly a purely blue-sensitive reaction and secondly a reaction with an action spectrum having peaks in the blue as well as in the red part of the spectrum[13]. A brief illumination of a coleoptile of *Avena* has a great influence on the protoplasmic streaming of the cells[12]. High intensities of light cause an acceleration in the flow, while retardation follows a brief illumination with rather low intensities. The first effect of the irradiation can be measured about 3–4 min after the short light impulse. In eliciting changes of this kind in *Avena* coleoptiles blue light is the most effective; thereafter comes violet and ultraviolet. The effect of green light and of light of longer wavelengths is negligible. The timing of these changes is very similar to those in the case of plasma viscosity.

Inducement of streaming by light has been known for a long time and there are observations concerning this effect of light in a series of older investigations[13]. The interesting fact here is that both blue and red light seem to be effective. Although red light is able to induce protoplasmic streaming, the streaming is not maintained if red light irradiation is continued. However, streaming does continue if small amounts of blue light are added. Thus long-wave light only acts as a transient stimulus while blue light acts as a stronger transient stimulus and also as a stationary one.

Alterations in permeability are important among other responses which seem to be elicited by blue light. Although light-induced permeability changes have not been so well investigated by modern techniques, it is evident that on the whole they follow the changes in protoplasmic viscosity. On the other hand light effects on ion uptake seem to be coupled to photosynthetic processes[14].

From this survey it is evident that we know of several responses of the single cell which are elicited by the same kind of light that causes macroscopically visible reactions of the plant. Whether they are parts of this macroscopic reaction or phenomena appearing at the same time as these but not correlated to them is, however, not yet established. A more accurate determination of the action spectra for the single processes than it has hitherto been possible to perform is probably necessary before the question whether connections exist can be answered.

References p. 26

In the phototropic response indolacetic acid is the active agent which causes the changes in growth rate. It is now known that this substance affects the viscosity of the cytoplasm[15] as well as protoplasmic streaming[16]. One can therefore not exclude the possibility that the effect of light upon these cell properties can go via changes in hormone contents. The causality of these effects of light is thus difficult to decide and many problems await solution.

In the general survey of the main blue-light responses which has been presented here, the phenomena have been treated as isolated processes not correlated to other light-responses of the plant, *e.g.* the red-light response. Until a few years ago the different light-dependent reactions were considered to work more or less independently of each other, but new experiments point to connections between the two systems.

Macroscopically, the purely blue-sensitive system seems mainly to be localized to the apical parts of the plant, parts which do not visibly respond to red light. It is evident however that we have a blue-sensitive system in all cells of the plant. This is also the case with the red responses. We might have macroscopic responses in only certain parts of plants although it can probably be shown that all cells respond in some way to red light.

It has recently been shown that some blue-light effects can be counteracted by far-red light although it should be emphasized that no purely phototropic reaction has yet been nullified by such light. Far-red light is not able to bring about changes in viscosity.

REFERENCES

1 James H. C. Smith, *Proc. 2nd Intern. Congr. Photobiol.*, 1958, p. 333.
2 R. B. Withrow, John B. Wolff and L. Price, *Plant Physiol.*, 31 (Suppl.) (1956) xiii.
3 Hemming I. Virgin, *Physiol. Plantarum*, 11 (1958) 347.
4 C. S. French and Violet K. Young, *J. Gen. Physiol.*, 35 (1952) 873.
5 W. Simonis, in *Handb. d. Pflanzenphysiol.*, ed. W. Ruhland, Vol. II, Springer, Berlin, 1956, p. 655.
6 Hans Mohr, *Ergeb. Biol.*, XXII (1960) 67.
7 R. B. Withrow and C. C. Moh, *Radiation Research*, 5 (1957) 491
8 Arthur W. Galston, in *Handb. d. Pflanzenphysiol.*, ed. W. Ruhland, Vol. XVII, Springer, Berlin, 1959, p. 492.
9 Hemming I. Virgin, *Physiol. Plantarum*, 5 (1952) 575
10 Hemming I. Virgin, *Physiol. Plantarum*, 4 (1951) 255.
11 Hemming I. Virgin, *Physiol. Plantarum*, 7 (1954) 343.
12 H. P. Bottelier, *Rec. trav. botan. néerl* , 31 (1934) 474
13 Noburô Kamiya, *Protoplasmic streaming* - · *Protoplasmatologia*, ed. L. V Heilbrunn and F. Weber, Vol. VIII, 1959, p. 3a.
14 L. Brauner, *Handb. d. Pflanzenphysiologie*, ed. W. Ruhland, Vol II, Springer, Berlin, 1956, p. 381.
15 M. G. Stålfelt, *Exptl. Cell Research, Suppl.* 1 (1949) 63.
16 B. M. Sweeney and K. V. Thimann, *J. Gen. Physiol.*, 25 (1942) 841.
17 M. G. Stålfelt, *Protoplasma*, XLV (1955) 285.
18 R. B. Withrow, *A Kinetic Analysis of Photoperiodism, Photoperiodism and Related Phenomena in Plants and Animals*, ed. Robert B. Withrow, Washington, 1959

LIGHT-INDUCED PHOSPHORYLATION

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I. A BIOCHEMICAL VIEW OF PHOTOSYNTHESIS

The concept is firmly established in cellular physiology that adenosine triphosphate (ATP) is a universal "energy-currency" acting as a link between energy-yielding and energy-consuming metabolic reactions. The cell as a chemical machine acts like man-made machines in that it runs only on one form of energy. An electric motor runs only on electricity, a steam engine on steam. Other fuels such as wood, coal, or oil must first be converted into steam or electricity before they can activate a steam engine or an electric motor. Likewise, in the living cell the chemical energy that is released by the breakdown of foodstuffs must first be converted into chemical energy that is stored in the pyrophosphate bonds of ATP, before it can be used for the multitude of energy-requiring processes on which life depends.

It is now well-known that ATP plays a key role as an energy transmitter in muscular contraction, in the active transport of solutes across semipermeable membranes, in glandular secretion, in the generation of electric current in nerve tissue (and in the electric organ of the eel), and in light emission by the firefly. What is of special concern to us here, however, is that ATP is required for the synthesis of carbohydrates, fats, proteins, and other essential cellular constituents.

Carbohydrates, as is well known, are the main products of photosynthesis in green plants. Two mechanisms have been invoked to explain the role of light in the formation of carbohydrates from CO_2 and water during photosynthesis. One postulation, ascribed to light the role of generating from water a reductant (H), with a reducing potential sufficiently strong to reduce CO_2 to the level of carbohydrates by some mechanism that is peculiar to photosynthesis and different from the known biochemical reactions for the dark assimilation of CO_2. This hypothesis has thus far remained speculative.

The alternative view regarded CO_2 assimilation in photosynthesis as being a dark chemosynthetic process that is fundamentally a reversal of the breakdown of carbohydrates which occurs in fermentation and respiration. The synthesis of carbohydrate by this pathway requires ATP and reduced pyridine nucleotide (PNH_2). According to this view, the distinction between photosynthetic and non-photosynthetic cells lies in the manner in which ATP and reduced pyridine nucleotide are formed. Photosynthetic cells form these compounds at the expense of light energy, whereas non-photosynthetic cells form them at the expense of energy released by dark reactions.

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References p. 42/43

CO_2 assimilation proper occurs, both in photosynthetic and non-photosynthetic cells, by dark reactions and is therefore removed from the domain of photochemical reactions (Fig. 1).

Experimental support for the second theory, *i.e.*, the biochemical view of CO_2 assimilation in photosynthesis, came from several directions (literature reviewed in ref.[1]). However, this interpretation of photosynthesis could not be drawn with finality without an investigation of the pathway of CO_2 assimilation at the particular cellular

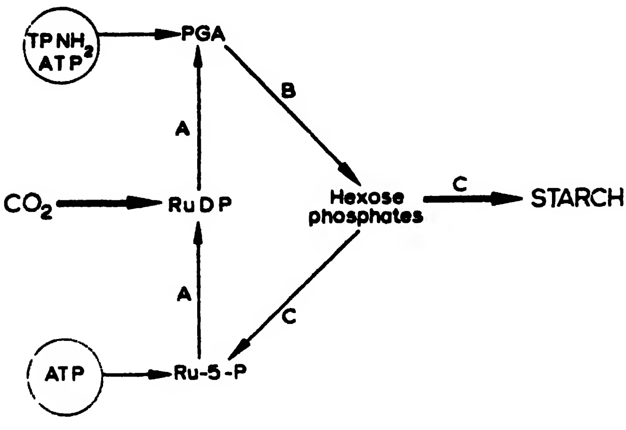

Fig. 1. Condensed diagram of the reductive carbohydrate cycle in chloroplasts. The cycle consists of three phases. In the carboxylative phase (A), ribulose-5-phosphate (Ru-5-P) is phosphorylated to ribulose diphosphate (RuDP) which then accepts a molecule of CO_2 and is cleaved to 2 molecules of phosphoglyceric acid (PGA); in the reductive phase (B) PGA is reduced and converted to hexose phosphates; in the regenerative phase (C) hexose phosphate is converted into storage carbohydrates (starch) and into the pentose monophosphate needed for the carboxylative phase. All the reactions of the cycle occur in the dark. The reactions of the carboxylative and reductive phases are driven by ATP and $TPNH_2$ formed in the light

site where sugar synthesis occurs in green plants. This site is now known to be the chloroplast[2-20] — a view that was once widely held without the support of critical experimental evidence[21,22] and was later abandoned because of evidence to the contrary[24-27], only to be formulated anew on the basis of experiments with tracer carbon and improved methods for isolating functional chloroplasts from leaves[2-20]. Recent experiments have also shown that within the chloroplast itself, the enzymes for CO_2 assimilation are localized in the water-soluble portion and are capable of carrying out the synthesis of sugar in the dark, even when they are physically separated from the chlorophyll-containing particles[7].

2. PHOTOSYNTHETIC PHOSPHORYLATION

The experiments with isolated chloroplasts have narrowed down the role of light in photosynthesis to those reactions in which reduced pyridine nucleotide and ATP are formed by light. With respect to reduced pyridine nucleotide it has already been shown by several laboratories that isolated chloroplasts were capable of reducing TPN to $TPNH_2$ in light, with a simultaneous evolution of oxygen[28-30]. What remained to be determined was the source of ATP in photosynthesis, or more specifically, the cellular site and the mechanism by which ATP is being formed during photosynthesis. From the standpoint of cellular physiology, the important question was whether the ATP used in photosynthesis was supplied by some light-driven assimilation of inorganic phosphate that is peculiar to photosynthesis, or whether the ATP used in photosynthesis was supplied by the process of respiration.

Before the recent investigations with isolated chloroplasts the only cytoplasmic

particles known to form ATP were mitochondria during the oxidative phosphorylation phase of respiration. Oxidative phosphorylation by mitochondria has therefore usually been invoked in explaining the source of ATP used in photosynthesis as, for example, by Calvin's group [*cf.* ref.[31], Fig. 7]. A model was proposed that envisaged the formation of ATP during photosynthesis by a collaboration between chloroplasts and mitochondria. Chloroplasts were supposed to form, in the light, $DPNH_2$, the "fuel" for oxidative phosphorylation, and $DPNH_2$ was then passed on to mitochondria, which reoxidized it with molecular oxygen during oxidative phosphorylation, generating in this manner ATP[32].

This model for the generation of ATP in photosynthesis posed a serious difficulty. The most specialized photosynthetic tissue, the mesophyll cells of leaves, are noted for their paucity of mitochondria. Within the mesophyll cells, especially in the part known as palisade parenchyma, chloroplasts are the dominant cytoplasmic bodies; mitochondria are few[33,34]. (This is not the case in less specialized plant cells.) It was difficult, therefore, to visualize how oxidative phosphorylation by so few mitochondria could generate enough ATP in tissues that have become especially adapted for vigorous photosynthetic activity.

The difficulty was removed when we discovered in 1954, that isolated chloroplasts are capable of synthesizing ATP in light without the aid of mitochondria[2]. When conditions were so arranged that CO_2 assimilation was excluded, isolated chloroplasts used light energy for the conversion of inorganic phosphate into ATP by esterifying adenosine diphosphate (ADP) with orthophosphate. It now became clear that it was unnecessary for chloroplasts to collaborate with mitochondria by making first a cellular substrate at the expense of light energy and then having the mitochondria convert this substrate to ATP. Light energy could be converted directly into ATP by chloroplasts alone in accordance with the overall reaction:

$$n \cdot P + n \cdot ADP \xrightarrow[\text{chloroplasts}]{\text{light}} n \cdot ATP \tag{1}$$

Light-induced ATP formation in chloroplasts raised at once the question whether this process is analogous to oxidative phosphorylation by mitochondria. Two fundamental differences were apparent. ATP formation by illuminated chloroplasts occurred without the consumption of molecular oxygen and without the addition of an external chemical substrate to supply free energy needed for the formation of the pyrophosphate bonds of ATP. The term photosynthetic phosphorylation[2,3] was therefore given to the light induced ATP formation by chloroplasts to distinguish it from oxidative (respiratory) phosphorylation by mitochondria and the anaerobic phosphorylations at substrate level that occur in glycolysis. In both of these processes ATP formation occurs at the expense of energy liberated by the oxidation of a chemical substrate, whereas the only "substrate" which is being consumed in photosynthetic phosphorylation is light.

3. PHOTOSYNTHETIC PHOSPHORYLATION IN CHLOROPLASTS AND BACTERIA

In assessing the role of photosynthetic phosphorylation in photosynthesis an important question was whether this phenomenon was peculiar to chloroplasts or whether it was also found in photosynthetic cells that do not have chloroplasts. Of special interest was the occurrence of photosynthetic phosphorylation in photosynthetic bacteria.

References p. 42/43

These cells do not have chloroplasts, and carry on photosynthesis anaerobically, oxygen being neither produced nor consumed. What was the role of oxygen in photosynthetic phosphorylation?

Oxygen seemed to be needed in some puzzling way for photosynthetic phosphorylation. The process when first discovered, proceeded at a sustained rate only in the presence of oxygen[2] (Fig. 2b); but a fundamental difference remained between this oxygen effect and the role of oxygen in oxidative phosphorylation by mitochondria. In photosynthetic phosphorylation oxygen acted as a catalyst, *i.e.*, there was no net consumption of oxygen in the course of the reaction. Purified chloroplasts were unable to form ATP in the dark by oxidizing known substrates of oxidative phosphorylation with molecular oxygen[35].

Further investigation of photosynthetic phosphorylation by spinach chloroplasts soon resulted in the identification of FMN and vitamin K as catalysts in the process (see ref.[36], p. 6326), [37,38]. At optimal but still catalytic concentrations of either FMN[38] or vitamin K, photosynthetic phosphorylation became independent of external oxygen and proceeded vigorously in an atmosphere of nitrogen or argon. At a much lower, "microcatalytic," concentration of the added cofactors, photosynthetic phosphorylation remained dependent on oxygen, although still showing no net oxygen consumption.

These findings are also in agreement with the recent results of Wessels[39], Jagendorf and Avron[40] and Nakamoto, Krogmann and Vennesland[41], that photosynthetic phosphorylation with suboptimal amounts of cofactors was oxygen-dependent but became oxygen-independent at higher concentrations of cofactors.

In charting our subsequent investigations, our group laid special stress on the anaerobic photosynthetic phosphorylation which proceeds in isolated chloroplasts at optimal catalytic concentrations of FMN and vitamin K. We considered this type more fundamental to photosynthesis in general than the oxygen-catalyzed type because it would also apply to ATP formation in bacterial photosynthesis in which oxygen cannot be involved.

Soon after the discovery of photosynthetic phosphorylation in isolated chloroplasts, Frenkel[42] reported a similar phenomenon in the photosynthetic bacterium *Rhodospirillum rubrum*. Although Frenkel suggested that the light-induced ATP formation in bacterial preparations was similar to that in chloroplasts, the similarity seemed uncertain at first, because Frenkel's photophosphorylation system, which was a sonic macerate of *R. rubrum* cells, differed in several respects from its counterpart in isolated chloroplasts. Frenkel's preparations became substrate-dependent after washing; the rate of photophosphorylation was doubled on adding α-ketoglutarate[42]. But in later experiments he ruled out the dependence on an added chemical substrate[43] and the equivalence of chloroplast and bacterial photophosphorylation seemed probable.

Frenkel's findings were followed by those of Williams[44] who demonstrated photosynthetic phosphorylation in cell-free preparations of the obligately anaerobic photosynthetic bacteria, *Chromatium* and *Chlorobium*. It thus became clear that a common *anaerobic* mechanism for a light-induced phosphorylation that does not depend on an exogenous chemical substrate or on oxygen consumption, is shared by both green plants and photosynthetic bacteria. The energy conversion process proper seemed to be fundamentally independent of oxygen although it was still possible that details of mechanisms were different in green plants and photosynthetic bacteria.

The discovery of photosynthetic phosphorylation in chloroplasts by Arnon *et al.*[2] and in bacterial particles by Frenkel[42] was confirmed and extended in a number of laboratories. Photosynthetic phosphorylation in isolated chloroplasts was observed by Avron and Jagendorf[45,46], Wessels[47], Vennesland and her associates[48,41]; in algae by Thomas and Haans[49] and Petrack[50]; and in photosynthetic bacteria by Geller and Gregory[51], Geller[52], Kamen and Newton[53] and Anderson and Fuller[54]. Whatley *et al.*[11,12] have also shown that photosynthetic phosphorylation by chloroplasts, which had previously been almost entirely limited to observations on chloroplasts isolated from one species, *viz.*, spinach, is also demonstrable with chloroplasts isolated from several other species of higher plants. It now seems well established, therefore, that the cytoplasmic particles, which in all photosynthetic organisms contain the chlorophyll pigments, also contain a closely bound phosphorylating system.

Soon after the demonstration of photosynthetic phosphorylation in isolated chloroplasts attempts were made to compare its rate with that of CO_2 assimilation by illuminated whole cells. Since, as with most newly-discovered cell-free reactions, the rates of photosynthetic phosphorylation were rather low, there was little inclination at first to accord this process quantitative importance (ref.[55] pp. 292, 345) as a mechanism for converting light into chemical energy.

With further improvement in experimental methods we obtained rates of photosynthetic phosphorylation up to 170 times higher[56] than those originally described[2] and even these high rates were exceeded by Jagendorf and Avron[57]. The improved rates of photosynthetic phosphorylation were equal to or greater than the maximum known rates of carbon assimilation in intact leaves. It appeared, therefore, that isolated chloroplasts retain, without substantial loss, the enzymatic apparatus for photosynthetic phosphorylation — a conclusion which was in harmony with parallel evidence that the phosphorylating system is tightly bound in the water-insoluble grana portion of the chloroplasts (*cf.*[1]).

4. THE ELECTRON FLOW MECHANISM OF PHOTOSYNTHETIC PHOSPHORYLATION

Anaerobic photosynthetic phosphorylation has provided direct experimental evidence for the view that the conversion of light into chemical energy is independent of the classical manifestations of photosynthesis in green plants: CO_2 reduction and oxygen evolution. The sole product of the anaerobic photosynthetic phosphorylation is ATP and the salient fact which must be explained is that a "high-energy" pyrophosphate bond is formed at the expense of absorbed light energy. There is no need, ***a priori***, to connect this reaction either with photolysis of water or with reduction of CO_2.

The simplest hypothesis to account for the formation of ATP in photosynthetic phosphorylation is to assume that, as in the dark phosphorylations of glycolysis and respiration, the formation of a pyrophosphate bond is also coupled with a release of free energy which occurs during electron transport, *i.e.*, when an electron drops from the higher energy level (that is has when it resides in the electron donor molecule) to the lower energy level that it assumes on joining the electron acceptor molecule. But, a mechanism for photosynthetic phosphorylation must also account for its unique features: ATP is formed without the consumption of an exogenous electron donor and electron acceptor. Unlike oxidative phosphorylation, photosynthetic phosphorylation consumes neither exogenous substrate nor molecular oxygen, only light energy.

References p. 42/43

A mechanism for photosynthetic phosphorylation must, therefore, provide for the generation of both an electron donor and an electron acceptor in the primary photochemical act when radiant energy is absorbed by chlorophyll. Investigations of photosynthesis at the cellular level, in which the main preoccupation has usually been with CO_2 assimilation and oxygen evolution, led to no cogent theory of the primary act of photosynthesis that would fit the experimental facts of photosynthetic phosphorylation. As summed up recently by Livingston, "physiologists and biochemists appear to believe that this question (the primary act of photosynthesis) was answered long ago by physicists while physicists find the problem distressingly complicated and therefore uninteresting"[58].

The mechanism of photosynthetic phosphorylation that we have proposed[59] over a year ago regards the photosynthetic particle, chloroplast or bacterial chromatophore, as a "closed" catalytic system. We have suggested that during the primary photochemical act, one component of the "closed" system, chlorophyll (bound to protein), becomes excited on absorbing a photon and "expels" one of its electrons that has been raised to a higher energy level. The excited chlorophyll thus becomes the electron donor. On losing an electron, chlorophyll assumes an "oxidized" state, and in this way also becomes the electron acceptor in photosynthetic phosphorylation. The key reaction in the proposed mechanism, the transfer of an "excited" electron from chlorophyll to a cofactor of photophosphorylation, is based on a type of reaction in photo chemistry that was experimentally documented by Lewis and Lipkin[62].

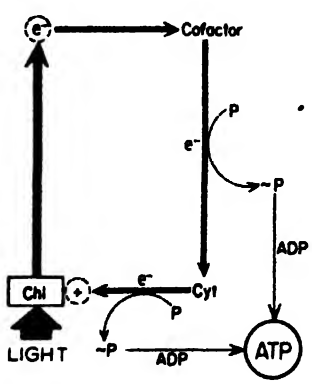

Fig. 2. Scheme for anaerobic cyclic photophosphorylation catalyzed by vitamin K_3 or FMN. Details in the text

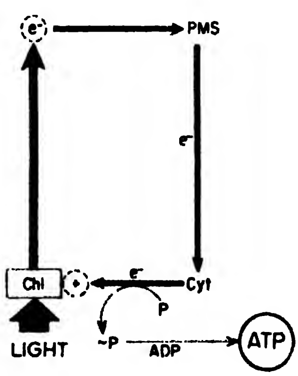

Fig. 3. Scheme for anaerobic cyclic photophosphorylation catalyzed by phenazine methosulfate (PMS). Details in the text.

The "expelled" electron returns in a stepwise manner to the "oxidized" chlorophyll molecule which thereupon resumes its normal ground state. On its return "downhill" path, the expelled electron releases free energy as it passes through several electron carriers. The electron carriers are the cofactors vitamin K, FMN, (or related physiological equivalents) and cytochromes. These intermediate electron carriers are coupled with enzyme systems that catalyze the phosphorylation process during which electron energy is converted into the pyrophosphate bond energy of ATP. A diagrammatic representation of this concept is given in Fig. 2.

Certain non-physiological agents, such as the dye phenazine methosulfate, have also been found to catalyze photosynthetic phosphorylation in bacterial chromato

phores[52,53] and in chloroplasts and presumably act by providing artificial "shortcuts"[52] for the physiological electron pathway from excited chlorophyll to cytochrome (Fig. 3). (Phenazine methosulfate has been shown by Massey[63] to be a very effective electron carrier in reactions involving cytochromes). At high light intensity the rate of cyclic photophosphorylation with phenazine methosulfate exceeds that catalyzed by vitamin K or FMN. There is evidence[1] for interpreting this as being the result of phenazine methosulfate by-passing a slow step in the process.

At low light intensity the overall rate of cyclic photophosphorylation is limited by the electron flow. Under these conditions the rate of photophosphorylation obtained with vitamin K or FMN as catalysts was about twice that obtained with phenazine methosulfate[1], suggesting that with these physiological cofactors more phosphorylating sites were operative (*cf.* Fig. 2 with Fig. 3).

The stepwise interaction of an electron, "activated" by light, with the intermediate electron acceptors in chloroplasts or chromatophores constitutes the energy conversion process in photosynthetic phosphorylation. Because of the cyclic path traveled by the "activated" electrons we have named this type of photosynthetic phosphorylation *cyclic photophosphorylation*[61,59]. In cyclic photophosphorylation electrons flow from chlorophyll to a cofactor, from the cofactor to cytochromes and from cytochromes back to chlorophyll. During this cyclic flow of electrons the physiological electron carriers present in the photosynthetic particles undergo oxidation-reductions that are coupled to phosphorylation reactions which produce ATP.

The proposed mechanism for cyclic photophosphorylation may be divided into three phases: (*a*) the light-induced generation of endogenous electron donor and endogenous electron acceptor (*b*) electron transport from the donor to the acceptor via a photosynthetic electron transport chain and (*c*) phosphorylation reactions coupled to electron transport. Phases (*b*) and (*c*) are analogous and possibly identical in some respects with their counterparts in oxidative phosphorylation whereas phase (*a*) is peculiar to photosynthetic phosphorylation.

5. EVIDENCE FOR ELECTRON FLOW MECHANISM IN CYCLIC PHOTOPHOSPHORYLATION

The validity of the proposed mechanism for cyclic photophosphorylation is supported by several lines of evidence which have been discussed elsewhere[59,1]. They will be briefly reviewed here.

In the absence of chloride, isolated chloroplasts lost their ability to evolve oxygen[64] but retained the capacity for anaerobic cyclic photophosphorylation[65,59,1]. Without chloride, isolated chloroplasts behaved like bacterial chromatophores: their photochemical activity was limited to anaerobic cyclic photophosphorylation.

In the absence of chloride, cyclic photophosphorylation itself was abolished, both in chloroplasts and chromatophores, when the electrons generated in the primary photochemical act were trapped by ferricyanide, and in this manner were prevented from completing the cyclic electron path that is coupled with ATP formation[65,59,1].

The important role which we assign to cytochromes as electron carriers in photophosphorylation is supported by a number of investigations in other laboratories (literature reviewed in refs.[59,1]) and by the evidence illustrated in Figs. 4 and 5. Fig. 4 shows successive oxidation of *Chromatium* cytochromes in light and reduction in the dark, in the same cell-free preparation.

Under the experimental conditions in which the *Chromatium* cell-free system was

References p. 42/43

investigated, the light-dark reversible oxidation-reduction reactions were sufficiently decelerated to be conveniently measurable with a recording spectrophotometer at room temperature[66].

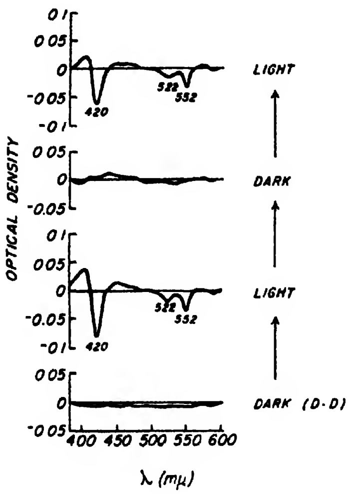

Fig. 4. Successive oxidation by light and reduction in the dark of cytochromes in cell-free preparations of *Chromatium*. The reaction mixture included in a final volume of 3.0 ml, chromatophores (P) containing 0.06 mg bacteriochlorophyll and supernatant fluid (S) corresponding to 0.5 mg bacteriochlorophyll. A small amount of $Na_2S_2O_4$ was previously added to S, which was then dialyzed against 0.2 *M* tris buffer, pH 7.8, prior to use. The reaction was carried out at room temperature. Gas phase argon. Difference spectra, using the dark treatment as control, were made in Thunberg type cuvettes, with a Cary recording spectrophotometer Illumination was by a tungsten lamp (35,000 Lux). (Nozaki, Ogata and Arnon[66]).

Chance and Nishimura[67] have recently found that, in whole *Chromatium* cells, a light-induced oxidation of cytochrome c_2 is independent of temperature. Furthermore, Arnold and Clayton[68], on illuminating bacterial chromatophores, observed temperature-independent (1°K to 300°K) reversible spectral changes near the absorption bands of bacteriochlorophyll. These findings accord with the main postulate of our theory[59] that the primary photochemical act in photosynthesis consists of electronic excitation and is thus independent of a thermal reaction.

Fig. 5 shows that the dark reduction of bacterial cytochromes, previously oxidized by light, was accelerated by vitamin K, the cofactor of cyclic photophosphorylation that is effective in bacterial photophosphorylation with aged chromatophores (see ref.[1], Table 2). Vitamin K was effective as an electron carrier when added either in its oxidized (vitamin K_3, 2-methyl-1,4-naphthoquinone) or reduced (vitamin K_5, 2-methyl-4-amino-1-naphthol hydrochloride) form but its effectiveness depended on the presence of chromatophores. Without chromatophores, using a purified cytochrome c_2, a hundred-fold greater concentration of reduced vitamin K was required to reduce the oxidized cytochrome.

6. CYCLIC PHOTOPHOSPHORYLATION AS PRIMITIVE PHOTOSYNTHESIS

The photophosphorylating system is structurally bound to chlorophyll in the smallest particles that function as units in the absorption of light energy, *i.e.* the chromatophores of bacteria and the grana of the chloroplasts. The close structural association of the phosphorylating activity with the chlorophyll pigments suggests that the harnessing of light energy in photosynthesis is more closely associated with ATP formation than with CO_2 assimilation. The enzymes responsible for CO_2 assimilation are easily dissociable from grana in the case of chloroplasts[5, 7], and not even structurally joined together in the case of bacterial chromatophores[54,69]. This is in agreement with the view[69], that in the course of biochemical evolution, photosynth

sis first emerged as a process for converting light energy into ATP and this "primitive" photosynthesis became only later a process linked to CO_2 reduction.

In the conventional view of photosynthesis, the chemical energy formed by the conversion of absorbed light is always used for the reduction of CO_2. The case of

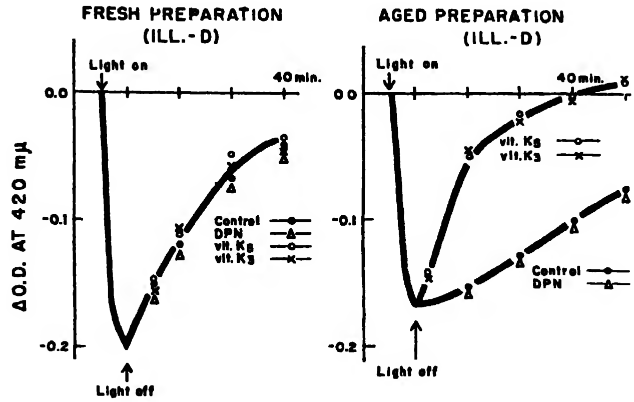

Fig. 5. Effect of vitamin K and other cofactors on the reduction of cytochromes c_2 in cell-free preparations of *Chromatium*. The cytochromes were oxidized by previous illumination (*cf.* Fig. 4). The reaction mixture included in a final volume of 3.0 ml, dialyzed cell-free suspension (PS) containing 0.06 mg bacteriochlorophyll, 0.02 μmoles of purified cytochrome c_2 and 0.03 μmoles of the respective cofactors. Difference in optical density was measured in a Beckman DU spectrophotometer with an attached photomultiplier using as controls cuvettes, with the respective cofactor omitted, in each case (Nozaki, Ogata and Arnon[66]).

cyclic photophosphorylation being a "primitive" photosynthesis in the evolutionary sense, would therefore be strengthened, if an example could be found today of a photosynthetic organism in which the only contribution of light to carbon assimilation was the formation of ATP.

Two such cases of photosynthesis in *Chromatium* have recently been described by Losada *et al.*[69]. In one case the sole source of carbon was acetate and in the other, CO_2. The photoassimilation of acetate occurred in the absence of an external hydrogen donor whereas in the photoassimilation of CO_2 the reductant was exogenous hydrogen gas. The sole contribution of light in both cases was the formation of ATP.

The experimental substitution of ATP for light was considered particularly significant because it was found in such photosynthetic bacteria as *Chromatium*, that are unique in the living world in being strict phototrophs. *Chromatium*, unlike, for example, *Chlorella* or photosynthetic bacteria of the genus *Rhodospirillum*, cannot replace its light-dependent mode of life by a heterotrophic, aerobic metabolism in the dark[70-72]. *Chromatium* grows only in the light[71], and being an obligate anaerobe, does not provide an alternative way for forming ATP by the mechanism of oxidative phosphorylation.

As regards the photoassimilation of acetate in another photosynthetic bacterium, the facultative anaerobe *R. rubrum*, a similar view that the contribution of light is limited to cyclic photophosphorylation was recently expressed, on the basis of independent evidence, by Stanier, Doudoroff, Kunisawa and Contopoulou[73].

In certain circumstances, ATP formation may be the sole contribution of light in the photosynthetic process, not only in bacteria but also in higher plants. We have

suggested elsewhere[61] that in green plants cyclic photophosphorylation may continue forming ATP when CO_2 assimilation is, for one reason or another, reduced or even stopped altogether. This might arise during the well-known midday closure of stomata in leaves of higher plants[74,75] which restricts the supply of CO_2. The closure of stomata often coincides with an abundance of starch and an incipient water deficit in the photosynthesizing cells. Under these conditions cyclic photophosphorylation, which consumes neither CO_2 nor water, would be a useful device for generating ATP to drive the many ATP-dependent reactions, notably the synthesis of polysaccharides, proteins and fats.

These theoretical deductions for higher plants have recently received experimental support from the work of Maclachlan and Porter[76]. They reported the first known instance of utilization of light energy in leaf tissue for the synthesis of starch from labelled glucose, under conditions when CO_2 assimilation was excluded but cyclic photophosphorylation could proceed.

7. THE PHOTOREDUCTANT IN BACTERIA

In the examples of "primitive" photosynthesis cited above, the only contribution of light was the formation of ATP. The second requirement for carbon assimilation that was discussed earlier, a supply of $DPNH_2$ or $TPNH_2$, was in the simplest case met by the inclusion of hydrogen gas. Photosynthetic, as well as other bacteria can use molecular hydrogen for reducing pyridine nucleotides in the *dark*, thus providing the $DPNH_2$ (or $TPNH_2$) that is needed for carbon assimilation (*cf.* ref.[1]).

Although photosynthetic bacteria when supplied with hydrogen gas do not require light energy for the production of $DPNH_2$ (or $TPNH_2$), a different situation arises when photosynthetic bacteria are grown with such hydrogen donors as for example, succinate or thiosulfate[70,71]. These hydrogen donors cannot reduce directly DPN (or

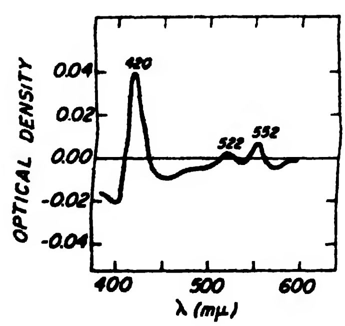

Fig. 6. Reduction of *Chromatium* cytochromes by thiosulfate in a cell-free system. Reaction mixture included, in a final volume of 3.0 ml of 0.1 *M* Tris buffer, pH 7.8, chromatophores (P) containing 0.1 mg bacteriochlorophyll and supernatant fluid (S) corresponding to 0.3 mg bacteriochlorophyll. 20 μmoles of thiosulfate were added to one of a pair of Thunberg-type cuvettes and the resulting difference spectrum was measured in a Cary spectrophotometer after 20 min at room temperature. Gas phase, argon. (Losada, Nozaki, and Arnon[77]).

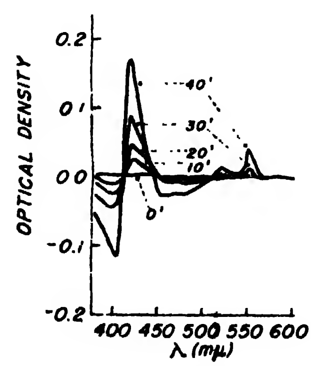

Fig. 7. Reduction of *Chromatium* cytochromes by succinate in a cell-free system. Reaction mixture included, in a final volume of 3.0 ml of 0.2 *M* Tris buffer, pH 7.8, chromatophores (P) containing 0.06 mg bacteriochlorophyll and supernatant fluid (S) corresponding to 0.3 mg bacteriochlorophyll. 10 μmoles of succinate were added to one of a pair of cuvettes and the resulting difference spectrum was measured in a Cary spectrophotometer at the indicated time intervals (Nozaki, Ogata, and Arnon[66]).

TPN) in the dark because the hydrogens or electrons which they donate have an insufficient reducing potential.

Additional energy is then required to bring about the reduction of DPN (or TPN) and, in a photosynthetic mode of life without oxygen that is characteristic of photosynthetic bacteria, this additional energy must come from light. If the electron flow mechanism is fundamental to the conversion of light into chemical energy, how can it apply to the photoreduction of pyridine nucleotides by thiosulfate or succinate?

The answer to this question came to us from recent experiments which demonstrated a reduction of bacterial cytochromes by thiosulfate and succinate. That cytochromes in photosynthetic cells are oxidized by light has been known from the work of H. Lundegardh in Sweden, L. N. M. Duysens in Holland, and B. Chance and coworkers in Philadelphia. Now we find that oxidized cytochromes are reduced by thiosulfate or succinate (Figs. 6 and 7). In this manner electrons donated by thiosulfate and succinate are transferred via cytochromes to chlorophyll and there raised at the expense of light energy to a reducing potential equal to that of molecular hydrogen[66,77].

The cytochrome systems in photosynthetic bacteria is thus a gateway through which electrons of a relatively low reducing potential enter and are "activated" *i.e.*, raised to a higher reducing potential at the expense of the energy of absorbed light. The fate of these "activated" electrons is different, however, than in the cyclic route. They are instead transferred by a "non-cyclic" electron flow mechanism to an external acceptor, of which three have been identified in parallel experiments: nitrogen gas, pyridine nucleotides, and protons. Preliminary evidence indicates that this non-cyclic electron flow is coupled with the formation of ATP (Fig. 8).

We found that the transfer of "activated electrons" to molecular nitrogen results in a photo-fixation of nitrogen gas — a result which we have now verified with isotopic nitrogen[78,79], *cf.* refs.[80,81]. In a parallel case, the transfer of "activated" electrons to pyridine nucleotides results in the photoreduction of DPN and TPN[82,83]. In still another case, the transfer of "activated" electrons to protons results in the photoproduction of hydrogen gas[84,85]. In all three cases thiosulfate or succinate is the electron donor. A consequence of these electron transfer reactions is the oxidation of thiosulfate to sulfate and succinate to fumarate[77,1].

In summary, there is experimental evidence for a non-cyclic electron flow in bacterial photosynthesis that supplements the cyclic electron flow mechanism which produces only ATP. The non-cyclic electron flow mechanism produces, without depending on a photolysis of water, electrons at a reducing potential at least equal to that of molecular hydrogen. We conclude that hydrogen gas is evolved in light when the "reducing" electrons become "surplus" because they are not consumed in metabolic reactions as, for example, in the photofixation of nitrogen gas or in the assimilation of CO_2 by forming reduced pyridine nucleotide.

8. NON-CYCLIC PHOTOPHOSPHORYLATION IN CHLOROPLASTS

We have seen that photosynthetic bacteria can reduce pyridine nucleotides in photosynthesis, either with molecular hydrogen in the dark or with certain electron donors, inorganic or organic, in the light. Green plants depend unconditionally on light for reducing pyridine nucleotide in photosynthesis because they use water as the

References p. 42/43

electron donor. The reduction of pyridine nucleotides with electrons donated by water requires a considerable input of energy which in photosynthesis is supplied by light.

As was mentioned earlier, isolated chloroplasts can reduce TPN in light with an accompanying evolution of oxygen. The relation of this reaction to photosynthetic phosphorylation seemed remote at first but recently, it was found to be very direct[61,60]. In the presence of ADP and inorganic phosphate, the photoreduction of TPN and oxygen evolution was coupled with the formation of ATP, in accordance with Reaction 2.

$$2\ \mathrm{TPN} + 2\ \mathrm{ADP} + 2\ \mathrm{P} + 2\ \mathrm{H_2O} \longrightarrow 2\ \mathrm{TPNH_2} + \mathrm{O_2} + 2\ \mathrm{ATP} \quad (2)$$

A similar photophosphorylation was observed when TPN was replaced by the non-physiological Hill reagent, ferricyanide[61,86]. With either TPN or ferricyanide the rate of oxygen evolution is greatly increased when it is coupled with phosphorylation. The conventional Hill reaction could thus be viewed as an uncoupled photophosphorylation, *i.e.*, a photochemical electron transport that is proceeding without its normally associated phosphorylation reaction[61,86].

The novel features of this new photophosphorylation reaction were first, that its ATP formation, unlike any other known phosphorylation, was coupled with an *evolution* of oxygen. Second, contrary to an analogy with oxidative phosphorylation, ATP formation resulted not in the ultimate oxidation but in the *reduction* of pyridine nucleotide.

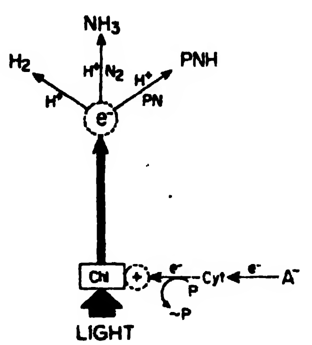

Fig. 8. Scheme for non-cyclic photophosphorylation in *Chromatium*. Details in the text

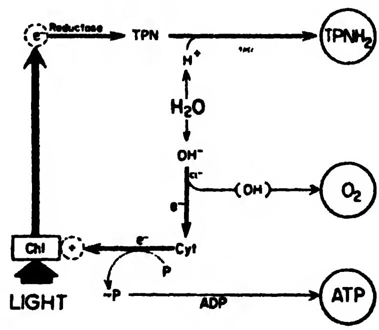

Fig. 9. Scheme for non-cyclic photophosphorylation in chloroplasts. Details in the text.

The mechanism of this reaction is visualized as a modification of the non-cyclic electron flow mechanism in photosynthetic bacteria to a stage at which *water* is the electron donor. The electrons from water reduce the cytochrome of chloroplasts that has been oxidized by light. We suggested that this is accomplished by an interaction between hydroxyl ions (or water) and a cytochrome component peculiar to the photosynthetic apparatus of green plants and absent in photosynthetic bacteria[59,1]. This reaction is visualized as being possibly an "anode"-type reaction, in which OH^- ions yield molecular oxygen and donate electrons, via a cytochrome chain, to chlorophyll where they are raised at the expense of absorbed light energy to the reducing potential required for TPN reduction. The formation of ATP is coupled with the oxidation of cytochrome. A corollary of the scheme is that water, though

not undergoing photolysis, is still the source of the oxygen evolved in photosynthesis by green plants (Fig. 9).

The proposed reactions leading to oxygen evolution appear to be thermodynamically feasible. The energy contribution of one einstein of red light, about 43 Kcal., is equivalent to a potential of 1.9V per faraday, and is sufficiently large, after making allowances for TPN reduction and ATP formation, to endow a chlorophyll-linked cytochrome with a redox potential more oxidizing than 0.815V, as is needed for oxygen evolution. However, no cytochrome having such properties has yet been demonstrated in chloroplasts.

In our present state of knowledge, the proposed mechanism for oxygen evolution must remain tentative. The possibility is not excluded that the transfer of electrons from OH^- to cytochromes may require an additional input of energy.

An important link in the chain of evidence connecting the photosynthetic events with the dark assimilation of CO_2 in chloroplasts was provided by experiments showing that both cyclic (Fig. 2) and non-cyclic photophosphorylation (Fig. 9) were essential[8]. The ATP formed in non-cyclic photophosphorylation alone is insufficient for the assimilation of CO_2 to the level of carbohydrates. Additional ATP must be supplied by cyclic photophosphorylation[9,10]. It is assumed that the intact cell has suitable regulatory mechanisms for keeping cyclic and non-cyclic photophosphorylation in balance.

9. OXYGEN-CATALYZED CYCLIC PHOTOPHOSPHORYLATION

The mechanisms of photosynthetic phosphorylation in chloroplasts discussed thus far include anaerobic cyclic photophosphorylation (Fig. 2) and non-cyclic photophosphorylation (Fig. 9). Recent work suggests the operation in chloroplasts of a third mechanism, an oxygen-catalyzed cyclic photophosphorylation[87].

As was already discussed above, we envisaged a catalytic role for oxygen in explaining the first experiments on photosynthetic phosphorylation, in which the presence of oxygen was required but no oxygen consumption was observed[12]. Interest in the role of oxygen was heightened when several laboratories reported recently that at low, "micro-catalytic" concentrations of FMN or vitamin K photophosphorylation remained dependent on oxygen[39–41].

From the standpoint of cellular physiology it was interesting to contrast the role of oxygen in ATP formation in photosynthesis with that in respiration. The participation of oxygen as the terminal electron acceptor in oxidative phosphorylation has conferred a marked superiority on respiration over fermentation, in the efficiency of converting the free energy of substrate into the energy of the pyrophosphate bonds of ATP. Was the efficiency of conversion of light energy into ATP also increased by the presence of oxygen?

Present evidence indicates that, in contrast to oxidative phosphorylation, the intervention of molecular oxygen in photosynthetic phosphorylation is an energy-wasteful step that lowers the efficiency of the anaerobic cyclic photophosphorylation process when light is limiting. The participation of oxygen may increase the overall rate of photophosphorylation in isolated chloroplasts only when light is abundant and phosphorylation is limited by a low concentration of cofactors[1].

On the basis of evidence now available, the participation of oxygen as a catalyst in cyclic photophosphorylation is represented by the diagram in Fig. 10. The proposed

References p. 42/43

mechanism, in which oxygen acts catalytically, provides for an exchange between molecular oxygen and the oxygen of water and is in agreement with ^{18}O exchange data recently reported by Nakamoto and Vennesland[88] and Jagendorf[89]. It appears that oxygen, when present in a system catalyzed by either FMN or vitamin K, is able to compete effectively with cytochromes for the electrons of cyclic photophosphorylation. Once the electrons are accepted by oxygen and form water, the cyclic pathway can be maintained only by a release of electrons in the oxygen-forming reaction of noncyclic photophosphorylation in chloroplasts (Fig. 9). By contrast, phenazine methosulfate catalyzes the transfer of electrons to cytochrome so effectively that it is able to prevent their "escape" to oxygen and hence the phenazine methosulfate pathway remains an "anaerobic" one, even when oxygen gas is present (*cf.* ref. [88]).

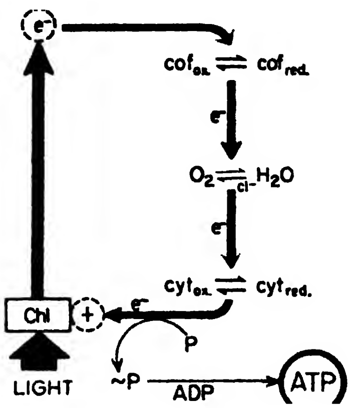

Fig. 10. Scheme for oxygen-catalyzed cyclic photophosphorylation in chloroplasts Details in the text.

10. PHOTOSYNTHETIC PHOSPHORYLATION AND BIOCHEMICAL EVOLUTION

The insight into the mechanism of photosynthesis, gained from the discovery of photosynthetic phosphorylation, permits us to interpret, with somewhat enhanced confidence, certain aspects of biochemical evolution.

The beginning of photosynthesis may be viewed as an emergence of a porphyrin that gave rise to chlorophyll and permitted the cell to use for metabolic purposes the energy of sunlight. This primitive photosynthesis consisted only of anaerobic cyclic photophosphorylation. This would be the case, for example, with such reduced carbon compounds as acetate that might have been present in the primitive environment. When CO_2 became the source of carbon no photochemically formed reductant was required as long as hydrogen gas was present in the atmosphere. Oparin[90] and Miller and Urey[91] have summarized the evidence that in the early periods of evolution of life forms, the environment contained simple carbon compounds such as acetate and hydrogen gas. This primitive type of photosynthesis is still seen today in photosynthetic bacteria. *Chromatium*, for example, is still capable of using molecular hydrogen for reducing in the dark the pyridine nucleotide that is needed for CO_2 assimilation or of photoassimilating acetate without the aid of external reductants.

The harnessing of light energy for the synthesis of ATP was an event of supreme importance to the cell. It provided the cell, in an *anaerobic* environment, with a

much more efficient mechanism than fermentation for the formation of ATP, which is the universal cellular "energy currency" needed in the transformation of existing carbon compounds, into fats, carbohydrates, proteins, etc. Cyclic photophosphorylation gave the *anaerobic* photosynthetic cell a mechanism which in efficiency of ATP formation is comparable with the process of oxidative phosphorylation in aerobic cells, that followed it later in the evolutionary scale.

From the point of view of biochemical evolution, one of the most interesting findings in cell-free photosynthesis was that higher, aerobic plants have retained to this day the anaerobic cyclic photophosphorylation as a mechanism for making ATP while sharing with other organisms in the acquisition of the process of oxidative phosphorylation by mitochondria.

Photosynthesis in *Chromatium* demonstrates that, in the absence of hydrogen gas, primitive photosynthetic cells generate electrons with a reducing potential equal to molecular hydrogen, from inorganic or organic electron donors such as thiosulfate or succinate. Light energy now serves a dual purpose. It supplies ATP by cyclic photophosphorylation and it provides electrons for reducing pyridine nucleotides.

Finally, in the most advanced type of photosynthesis, found in green plants, water became the electron donor. Here the function of light was again to provide ATP but also to raise the electrons from water to an energy level high enough for the reduction of TPN. Only in the last case, when water became the electron donor, did oxygen evolution form an inseparable part of photophosphorylation in chloroplasts (Fig. 9).

Non-cyclic photophosphorylation enabled green plants to form a CO_2 reductant at the expense of light energy with the aid of an ubiquitous substance, water, and in this way to invade and live autotrophically in areas devoid of reduced sulfur compounds or of other electron donors of restricted distribution. The resultant proliferation of plant growth on the surface of the earth was responsible for releasing to the atmosphere the oxygen, locked in the water molecule, by the only known important mechanism capable of accomplishing this, photosynthesis of green plants.

Once molecular oxygen became available, the way was open for biochemical evolution to progress toward aerobic metabolism. The oxygen-independent cyclic photophosphorylation by chlorophyll-containing particles could now be paralleled by an efficient biological utilization of the energy of chemical substrates through the mechanism of oxidative phosphorylation of mitochondria. Photosynthesis of green plants now provided both the substrates and oxygen to make oxidative phosphorylation and aerobic life on this planet possible.

11. ENERGY CONVERSION CONCEPT IN PHOTOSYNTHESIS

The concept of photosynthesis which emerges from the discovery of photosynthetic phosphorylation differs from the conventional view of photosynthesis that it is mainly a process of CO_2 assimilation. Photosynthesis appears to be first and foremost a process for converting sunlight into chemical energy and this conversion is more directly associated with phosphorus than with carbon assimilation. The trapped light energy is first converted into ATP and reduced pyridine nucleotides and then may be used by the cell for assimilation of carbon, nitrogen, phosphorus or sulfur compounds or for any other energy-requiring cellular activity. Photosynthesis may now be defined as the synthesis of cellular substances at the expense of chemical energy

References p. 42/43

formed by photochemical reactions. This definition includes, but is not limited to, CO_2 assimilation.

The photoassimilation of acetate by *Chromatium* is a case of photosynthesis without either oxygen evolution or CO_2 reduction. So is the light-dependent conversion of glucose into starch. In the conventional view of photosynthesis this state of affairs would be a contradiction of terms, but according to our present concept these examples represent photosynthesis because they involve biochemical syntheses that are being driven by light energy after light has been converted by the cell into ATP.

In this view of photosynthesis, CO_2 assimilation, although quantitatively the dominant form of photosynthesis on our planet, is fundamentally only a special case of the use and storage of light energy. CO_2 assimilation proper, in both green plants and photosynthetic bacteria, consists exclusively of dark reactions that are not peculiar to photosynthesis. The familiar accumulation of carbon compounds as carbohydrates during photosynthesis in green plants constitutes storage of trapped light energy. The first products of photosynthesis in green plants, ATP and $TPNH_2$, are present in the cell only in small, catalytic amounts and cannot be stored to any appreciable degree for future use. The main storage products of trapped light energy that sustain life on our planet are carbohydrates, fats and proteins.

REFERENCES

1 D. I. Arnon, in W. D. McElroy and B. Glass (Editors), *Symposium on Light and Life*, The John Hopkins Press, Baltimore, 1961, p. 489.
2 D. I. Arnon, M. B. Allen and F. R. Whatley, *Nature*, 174 (1954) 394
3 D. I. Arnon, *Science*, 122 (1955) 9.
4 M. B. Allen, D. I. Arnon, J. B. Capindale, F. R. Whatley and L. J. Durham, *J. Am. Chem. Soc.*, 77 (1955) 4149.
5 D. I. Arnon, M. B. Allen, F. R. Whatley, J. B. Capindale and L. L. Rosenberg, *Proc 3rd Intern. Congr. Biochem , Brussels, 1955*, Academic Press, New York, 1956, p. 227.
6 F. R. Whatley, M. B. Allen, L. L. Rosenberg, J. B Capindale and D. I. Arnon, *Biochim. Biophys. Acta*, 20 (1956) 462.
7 A. V. Trebst, H. Y. Tsujimoto and D I. Arnon, *Nature*, 182 (1958) 351
8 A. V. Trebst, M. Losada and D. I. Arnon, *J. Biol. Chem.*, 234 (1959) 3055.
9 M. Losada, A. V. Trebst and D. I. Arnon, *J. Biol Chem* , 235 (1960) 832.
10 A. V. Trebst, M. Losada and D. I. Arnon, *J. Biol Chem* , 235 (1960) 840.
11 F. R. Whatley, M. B. Allen, A. V. Trebst and D. I. Arnon, *Plant Physiol* , 33 (1958) (Suppl.) xxvii.
12 F. R. Whatley, M. B. Allen, A. V Trebst and D. I Arnon, *Plant Physiol* , 35 (1960) 188.
13 R. Ueda, *Botan. Mag. (Tokyo)*, 62 (1949) 731
14 L. R. Irmak, *Rev. Fac. Sci. Univ Istanbul*, Ser. B, 20 (1955) 237
15 J. B. Thomas, A. J. M Haans and A A. van der Leun, *Biochim Biophys. Acta*, 25 (1957) 153.
16 M. Gibbs and M. A. Cynkin, *Nature*, 182 (1958) 1241
17 N. E. Tolbert, *Brookhaven Symposia in Biol* , 11 (1958) 271
18 M. Gibbs and N. Calo, *Plant Physiol.*, 34 (1959) 318
19 R. M. Smillie and R. C. Fuller, *Plant Physiol.*, 34 (1959) 651.
20 R. M. Smillie and G. Krotkov, *Can. J Botan* , 37 (1959) 1217.
21 J. Sachs, *Lectures on the Physiology of Plants*, Clarendon Press, Oxford, 1887.
22 W. Pfeffer, *Physiology of Plants*, Clarendon Press, Oxford, 1900.
23 R. Hill, *Symposia Soc. Exptl. Biol.*, 5 (1951) 223.
24 A. H. Brown and J. Franck, *Arch. Biochem* , 16 (1948) 55.
25 A. A. Benson and M. Calvin, *Ann. Rev Plant Physiol.*, 1 (1950) 25.
26 E. Rabinowitch, *Ann Rev. Plant Physiol.*, 3 (1952) 229.
27 R. Lumry, J. D. Spikes and H. Eyring, *Ann Rev. Plant Physiol.*, 5 (1954) 271.
28 W. Vishniac and S. Ochoa, *Nature*, 167 (1951) 768.
29 L. J. Tolmach, *Nature*, 167 (1951) 946.
30 D. I. Arnon, *Nature*, 167 (1951) 1008.
31 J. A. Bassham, A. A. Benson, L. D. Kay, A. Z Harris, A. T. Wilson and M. Calvin, *J. Am. Chem. Soc.*, 76 (1954) 1760.
32 W. Vishniac and S. Ochoa, *J. Biol. Chem.*, 198 (1952) 501.

33 K. Esau, *Plant Anatomy*, J. Wiley and Sons, New York, 1953.
34 W. O. James and V. S. Das, *New Phytologist*, 56 (1957) 325.
35 D. I. Arnon, M. B. Allen and F. R. Whatley, *Biochim. Biophys. Acta*, 20 (1956) 449.
36 D. I. Arnon, F. R. Whatley and M. B. Allen, *J. Am. Chem. Soc.*, 76 (1954) 6324.
37 D. I. Arnon, F. R. Whatley and M. B. Allen, *Biochim. Biophys. Acta*, 16 (1955) 607.
38 F. R. Whatley, M. B. Allen and D. I. Arnon, *Biochim. Biophys. Acta*, 16 (1955) 605.
39 J. S. C. Wessels, *Biochim. Biophys. Acta*, 29 (1958) 113.
40 A. T. Jagendorf and M. Avron, *Arch. Biochem. Biophys.*, 80 (1959) 246.
41 T. Nakamoto, D. W. Krogmann and B. Vennesland, *J. Biol. Chem.*, 234 (1959) 2783.
42 A. W. Frenkel, *J. Am. Chem. Soc.*, 76 (1954) 5568.
43 A. W. Frenkel, *J. Biol. Chem.*, 222 (1956) 823.
44 A. M. Williams, *Biochim. Biophys. Acta*, 19 (1956) 570.
45 M. Avron and A. T. Jagendorf, *Nature*, 179 (1957) 428.
46 M. Avron, A. T. Jagendorf and M. Evans, *Biochim. Biophys. Acta*, 26 (1957) 262.
47 J. S. C. Wessels, *Biochim. Biophys. Acta*, 25 (1957) 97.
48 C. T. Chow and B. Vennesland, *Plant Physiol.*, 32 (Suppl.) (1957) iv.
49 J. B. Thomas and A. M. J. Haans, *Biochim. Biophys. Acta*, 18 (1955) 286.
50 B. Petrack, *Federation Proc.*, 18 (1959) 302.
51 D. M. Geller and J. S. Gregory, *Federation Proc.*, 15 (1956) 260.
52 D. M. Geller, doctoral dissertation, Division of Medical Science, Harvard University, 1957.
53 M. Kamen and J. W. Newton, *Biochim. Biophys. Acta*, 25 (1957) 462.
54 I. C. Anderson and R. C. Fuller, *Arch. Biochem. Biophys.*, 76 (1[illegible]58) 168.
55 H. Gaffron, ed., *Research in Photosynthesis*, Interscience, New York, 1957.
56 M. B. Allen, F. R. Whatley and D. I. Arnon, *Biochim. Biophys. Acta*, 27 (1958) 16.
57 A. T. Jagendorf and M. Avron, *J. Biol. Chem.*, 231 (1958) 277.
58 R. Livingst[illegible], [illegible] Pirson (Editor), *Handbuch der Pflanzenphysiologie*, Vol. 5, Springer, Heidelberg, Germany, 1960, p. 832.
59 D. I. Arnon, *Nature*, 184 (1959) 10.
60 D. I. Arnon, F. R. Whatley and M. B. Allen, *Biochim. Biophys. Acta*, 32 (1959) 47.
61 D. I. Arnon, F. R. Whatley and M. B. Allen, *Science*, 127 (1958) 1026.
62 G. N. Lewis and D. Lipkin, *J. Am. Chem. Soc.*, 64 (1942) 2801.
63 V. Massey, *Biochim. Biophys. Acta*, 34 (1959) 255.
64 O. Warburg, *Heavy Metal Prosthetic Groups and Enzyme Action*, Clarendon Press, Oxford, 1949, p. 213.
65 J. and C. Bové, F. R. Whatley and D. I. Arnon, Data presented by D. I. Arnon at *IXth Intern. Botan. Congress*, Montreal, Canada, August, 1959.
66 M. Nozaki, M. Ogata and D. I. Arnon, Reported by D. I. Arnon at *Symposium on Comparative Biochemistry of Photoreactive Pigments*, Pacific Grove, Calif., Sept. 1959.
67 B. Chance and M. Nishimura, *Proc. Natl. Acad. Sci. U.S.*, 46 (19[illegible]) 19.
68 W. Arnold and R. K. Clayton, *Proc. Natl. Acad. Sci. U.S.*, 46 (19[illegible]) 769.
69 M. Losada, A. V. Trebst, S. Ogata and D. I. Arnon, *Nature*, 186 ([illegible]60) 753.
70 C. B. van Niel, *Arch. Microbiol.*, 3 (1931) 1.
71 F. M. Müller, *Arkiv. Mikrobiol.*, 4 (1933) 131.
72 C. B. van Niel, in J. Franck and W. E. Loomis (Editors), *Photosynthesis in Plants*, Iowa State College Press, 1949, p. 437.
73 R. Y. Stanier, M. Doudoroff, R. Kunisawa and R. Contopoulou, *Proc. Natl. Acad. Sci. U.S.*, 45 (1959) 1246.
74 M. G. Stalfelt, *Physiologia Plantarum*, 8 (1955) 572.
75 O. V. S. Heath and B. Orchard, *Nature*, 180 (1957) 180.
76 G. A. Maclachlan and H. K. Porter, *Proc. Roy. Soc., (London)*, B 150 (1959) 460.
77 M. Losada, M. Nozaki and D. I. Arnon, in W. D. McElroy and B. Glass (Editors), *Light and Life*, Johns Hopkins Press, Baltimore, 1961, p. 510.
78 D. I. Arnon, M. Losada, M. Nozaki and K. Tagawa, *Biochem. J.*, 11 (1960) 23 P.
79 D. I. Arnon, M. Losada, M. Nozaki and K. Tagawa, *Nature* (in the press).
80 M. D. Kamen and H. Gest, *Science*, 109 (1949) 56
81 J. W. Newton and P. W. Wilson, *Antonie van Leeuwenhoek*, 19 (1953) 71.
82 A. W. Frenkel, *Brookhaven Symposia in Biol.*, 11 (1958) 276.
83 L. P. Vernon and O. K. Ash, *J. Biol. Chem.*, 234 (1959) 1878.
84 H. Gaffron and J. Rubin, *J. Gen. Physiol.*, 26 (1942) 219.
85 H. Gest and M. D. Kamen, *J. Bacteriol.*, 58 (1949) 239.
86 A. T. Jagendorf, *Brookhaven Symposia in Biol.*, 11 (1958) 236.
87 H. Y. Tsujimoto, D. O. Hall and D. I. Arnon, Unpublished data (1960).
88 T. Nakamoto and B. Vennesland, *Federation Proc.*, 19 (1960) 329.
89 A. T. Jagendorf, Personal communication (1960).
90 A. I. Oparin, *The Origin of Life on the Earth*, 3rd Ed., Academic Press, New York, 1957.
91 S. L. Miller and H. C. Urey, *Science*, 130 (1959) 245.

THE EFFECTS OF LONG VISIBLE AND NEAR INFRARED RADIATION ON PLANTS

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This paper is devoted to photochemical reactions other than photosynthesis which must occur in plants to enable "normal" growth and development. This interesting field of photobiology which we call "photomorphogenesis" (that means: the non-photosynthetic control of plant growth and development by light) has been intensely investigated during the last 8 to 10 years. The results of these recent investigations are fully as fascinating as the recent results in the field of photosynthesis. I shall try now to present a kind of condensed summary.

First we have to point out a basic difference between photosynthesis and photomorphogenesis. In photosynthesis radiant energy is converted into chemical energy. In photomorphogenesis the photochemical reactions have a controlling, or regulating function. They do not supply the metabolism with energy but they regulate in a specific way — which is determined by the cell's organisation — the metabolism of the cells and of the organism. This is the reason why in many cases a relatively small quantity of photomorphogenically effective radiation will produce drastic effects.

The phenomena of photomorphogenesis demonstrate that in many plants photochemical reactions occur — independently of photosynthesis — as a result of electronic excitations of pigment molecules. These photochemical reactions finally result in drastic changes in growth and development. With regard to the complex mechanism of photomorphogenesis we are able to ask the following questions: What type of pigment absorbs the effective radiation, what kinds of photochemical reactions occur, and what are the causal relationships between the photochemical reactions and the final photoresponses which we can easily observe (for instance, light-induced growth of the cotyledons of a seedling). This is, of course, a tremendous complex of questions and unfortunately we cannot answer many of them, but we do know at least a little.

In earlier years it was generally accepted that essentially only short wavelength visible light, the so called "blue light" was photomorphogenically effective. It was stated in practically all textbooks on plant physiology that only blue light can produce the "normal" appearance of white-light-grown plants. In other words: only blue light can act like white light. — We know today that this concept is completely wrong, for visible light of long wavelength can also be highly effective in photomorphogenesis. We have learned that many plants possess several photomorphogenic reaction systems and that both short and long wavelengths can influence the growth of plants, at least in the case of potentially green plants. The predominance of one or another of these

systems depends on the physiological state of the plant and on the time, length and intensity of the irradiation.

And now we shall try to make a few statements on the effect of long visible and near infrared radiation on plants. The photomorphogenesis of the potentially green plant is controlled by several photoreactive systems. We shall concentrate first on one reaction system which is very important for the plant and which is the best known at the moment. I mean the "reversible red, far-red photoreactive system". The pigment system involved is the so-called "red, far-red pigment system". It is -- as we know today — distributed in the plant kingdom in a similar way as chlorophyll (namely among the potentially green plants) and it has an importance as basic for photomorphogenesis as that of chlorophyll for photosynthesis[1,3,12].

The essential features of this reversible red, far-red system may be briefly illustrated by using — in a simplified manner - the classical example of induction of germination by light. Lettuce seeds of a light-sensitive variety are sown on a suitable medium at 25° and kept under white fluorescent light. After 24 hours all seeds have germinated. If you place the seeds in darkness after sowing practically no germination occurs. The seeds are obligate "light germinators". It can be shown that even a short illumination of the imbibed seeds (*e.g.* a few minutes with white fluorescent light of medium intensity) can induce germination. If one places the seeds in darkness after the light flash, complete germination occurs. One can determine now the action spectrum of this induction of germination (that means, the relative efficiency of the different parts of the spectrum). The results show that essentially only radiation between 550 and 700 mμ can induce germination. Radiation around 660 mμ is the most effective. We call this spectral range between 550 and 700 mμ "red". Radiation above 700 mμ does not induce germination. At first sight it seems that this radiation is without effect. But if you induce 100% germination with red light and then irradiate immediately after the red with a wave length above 700 mμ, for instance 730 mμ, no germination occurs. In other words, the induction of germination by red can be nullified by a subsequent irradiation with the wave length 730 mμ. The induction of germination with red is thus "reversible". If we determine the action spectrum of the reversing effect, we find radiation between about 700 and 800 mμ effective. The highest efficiency is around 730 mμ. We call this spectral range between 700 and 800 mμ "far-red" in spite of the fact that the longest wave lengths of this range are no longer visible.

I repeat the principal results at this point of the discussion: We can induce the germination of lettuce seed with red and we can reverse this induction by immediately following with far-red. Following the far-red with red again results in 100% germination -- and so on. In short, germination or inhibition of germination is determined by the type of radiation (red or far-red) which has been applied immediately before the seeds are put into darkness.

The short wavelength visible radiation below 550 mμ is relatively ineffective, compared with red or far-red. We can neglect this spectral range in our present considerations.

During the last few years it has been shown that this "reversible red, far-red reaction system" not only plays the deciding role in seed or spore germination but it has been found that this system is involved in a great number of photoresponses among potentially green plants from the algae, mosses and ferns up to the monocotyledons[3,12].

References p. 49

In all cases one can induce a photoresponse (for instance the enlargement of the leaves of a dark grown seedling) by red and one can nullify this induction by immediately following with far-red. In several cases the action spectra of the red and of the far-red action have been determined. The corresponding action spectra are very similar in spite of the fact that they have been worked out with very different organisms, for example cells of algae, spores of ferns, etiolated cotyledons or leaves of adult, green plants[3,12,17].

The practically universal occurrence of the reversible red, far-red reaction system among potentially green plants can be simply explained by the assumption that the same reversible red, far-red *pigment* system occurs in cells of all those organisms which demonstrate this reaction mechanism. You find a generalized formula for this reversible pigment system in Fig. 1. A pigment with the absorption peak in the red

$$P_r \underset{\text{far-red}}{\overset{\text{red}}{\rightleftharpoons}} P_{fr} \dashrightarrow \text{photoresponse}$$

Fig. 1. A generalized formula for the reversible red, far-red pigment system.

which we symbolize by P_r can be transformed by an irradiation with red into a pigment P_{fr} which has its absorption peak in the far-red. By absorbing far-red quanta the excited P_{fr} can be transformed back into P_r. P_{fr} is the physiologically active form of the reversible pigment system. It is probably an enzyme, the function of which in the metabolism of the cell finally leads to the photoresponse. The name "phytochrome" has been given to the reversible red, far-red pigment system.

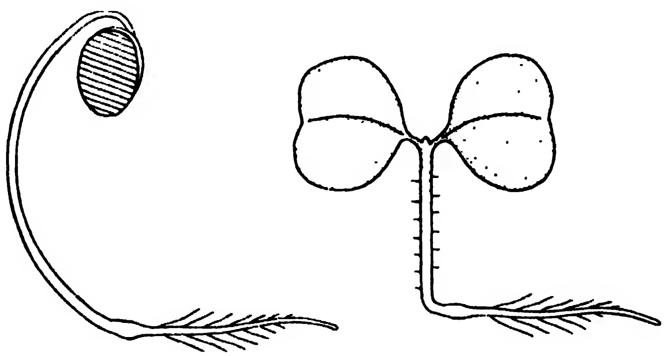
The photobiological experiments of recent years which have been performed with modern equipment and with much experimental skill led to excellent results which allowed a well-founded physico-chemical theory of the reversible red, far-red pigment system solely on the basis of *physiological* data[2,5].

However, for a long time, it was impossible to isolate the pigment system by physico-chemical means. The concentration of this pigment system *in vivo* is extremely small in spite of the fact that it exerts a very strong influence on growth and development of plants. During the past year the well-known photobiological team at Beltsville in the United States has been able to obtain the reversible red, far-red pigment system in cell-free solution and to observe — with aid of new instruments — the reversible transformation *in vitro* and *in vivo*[4]. It is, undoubtedly, only a question of time till we know more about the chemical nature of the pigment. It is a triumph of photobiology that the pigment system, the photochemistry of which can now be studied *in vitro* and *in vivo* is characterized by exactly those properties which were predicted years ago (especially by Hendricks) on the basis of the physiological data (for instance, it is a reversible chromoprotein; the photochemical reactions in both directions are of first order; P_r is stable in darkness, P_{fr} changes to P_r slowly in darkness at room temperature while at 3° there is no transformation in darkness; etc).

At normal temperatures (for instance, at 25°) and in darkness the pigment system is predominantly in the form P_r. We can assume, as we mentioned already, that P_{fr} is the "physiologically active" substance. The chromoprotein P_{fr} is apparently an enzyme. The enzymatic effect, the final consequence of which is the photoresponse,

continues as long as P_{fr} is present. When P_{fr} is transformed into P_r the enzymatic effect stops. We must imagine that the function of P_{fr} in the cell leads to a basic change in the cellular metabolism. But now it depends on the specific differentation of the cells and tissues which final photoresponse results. The practically exclusive presence of P_r in the cells is correlated with the etiolated appearance of the plant.

Let us consider a dicotyledonous seedling, for instance a mustard seedling, in Fig. 2. All the photoresponses in Fig. 2 can be induced by red, and in all cases the induction



Photoresponses:
Enlargement of cotyledons
Synthesis of anthocyanin
Opening of the plumular hook
Inhibition of hypocotyl lengthening
Hair formation along the hypocotyl
Increase of the negative geotropic reactivity

Fig. 2. (left) A schematic representation of a dark-grown seedling of mustard (*Sinapis alba* L.). 3 days after germination and (right) a corresponding light-grown seedling. All observable photoresponses can be induced by red, and in all cases the induction can be reversed with far-red.

can be reversed with far-red[9-11,13]. In other words, all photoresponses are results of the formation of P_{fr} in the cells of the seedling. The qualitatively quite different responses of the different organs of the seedling as a consequence of the same photochemical reaction have to do with the "specific differentiation" of the cells which compose the different organs of the organism. This "pattern of differentiation" is determined by the genotype of the plant and is built up in the course of the development of the seedling.

Besides the reversible red, far-red reaction system which can be demonstrated physiologically with only a small amount of effective radiation energy and which we therefore call a "low-energy reaction", another physiologically important photoreactive system has been found in recent years[9, ,15,16]. Apparently this second system is of great importance for photomorphogenesis of the potentially green plant under natural conditions of radiation. In many cases (for instance with *Sinapis alba*) the function of this second reaction system leads to the same photoresponses as the action of the reversible red, far-red system[11]. In this case of synergism both systems can be separated physiologically only with difficulty. But recently we investigated photoresponses of lettuce seedlings where the two systems function in an antagonistic way and can

therefore be separated physiologically without too much trouble. Because of the features of the action spectrum (with peaks in far-red and in blue) we call this second system the "blue, far-red reaction system". You see a representative action spectrum of the "blue, far-red reaction system" in the case of *Sinapis alba* in Fig. 3, together with the action spectrum of the induction of a photoresponse by the "low energy reaction". The function of this blue, far-red system can only be demonstrated in the laboratory when we irradiate the plant for a relatively long time with a rather high irradiance. Therefore we call this system the "high-energy reaction".

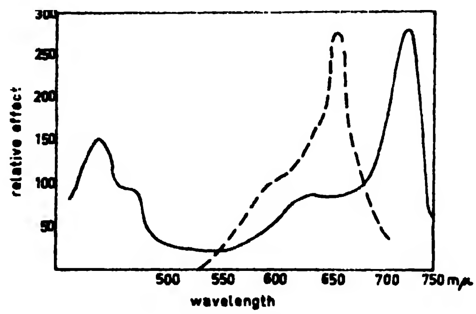


Fig. 3. An action spectrum (solid curve) of the "blue, far-red reaction system" (high energy reaction). The following photoresponse has been used: Light-induced enlargement of the cotyledons of mustard seedlings (*Sinapis alba* L.) The dashed curve represents the action spectrum for the induction of a photoresponse by the "low energy reaction".

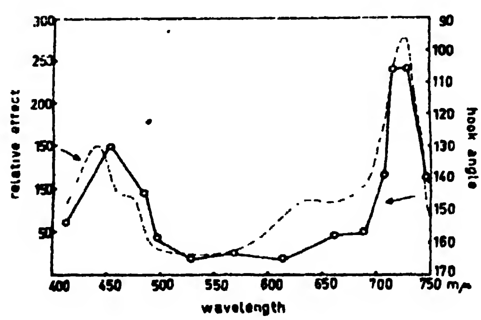


Fig. 4. An action spectrum (solid line, circles) for the reopening of the plumular hook in lettuce seedlings (*Lactuca sativa* L.) The action spectrum of the high energy reaction in mustard seedlings (Fig 3) is indicated by the dashed curve

An interesting example of the effectiveness of both systems may be briefly presented[14]. Dark-grown seedlings of lettuce do not form an appreciable plumular hook. But a "normal" plumular hook, as is formed *e.g.* by *Phaseolus vulgaris* in complete darkness, can be induced by red light. We could show that the *formation* of the plumular hook in lettuce seedlings is controlled exclusively by the reversible red, far-red reaction system ("low-energy reaction"). The hook which has been closed by red radiation can be reopened by light. Only blue and far-red radiation are effective in this respect. The hook can only be reopened if blue or far-red are applied with rather high irradiance over a relatively long period ("high-energy reaction"). The action spectrum (Fig. 4) and the kinetics of this high-energy reaction show that this photoreaction is identical

with the "blue, far-red reaction system" which we know, *e.g.* from the photomorphogenesis of mustard seedlings, and which apparently is also involved in many other photomorphogenic responses, as has been demonstrated by the Beltsville group and others. In mustard the low- and the high-energy reactions always function in a synergistic manner; in lettuce the opposite is true. The closing of the plumular hook is controlled by the reversible red, far-red system and the reopening of the hook is controlled by the blue, far-red system. In this way the two systems can be more clearly separated and characterized physiologically. It has been assumed by Hendricks *et al.*[6, 8] that the high-energy reaction is based on a sensitized photochemical reaction. The sensitizer involved is supposed to be the reversible red, far-red pigment system under conditions of a continued, simultaneous excitation of both pigments (P_r and P_{fr}). Under such steady state conditions part of the excitation energy is not used for reversible transformation but is transferred to an acceptor molecule. There is no space left to discuss in detail this rather complicated and highly speculative theory of the blue, far-red photomorphogenic reaction system, the investigation of which is going on in several laboratories. It has not so far been possible to demonstrate this "blue, far-red system" by photochemical or physico-chemical means.

REFERENCES

1 H. A. Borthwick, S. B. Hendricks and M. W. Parker, *Proc. Natl. Acad. Sci., U.S.*, 38 (1952) 929.
2 H. A. Borthwick, S. B. Hendricks, E. H. Toole and V. K. Toole, *Bot. Gaz.*, 115 (1954) 205.
3 H. A. Borthwick and S. B. Hendricks, in W. Ruhland (Editor), *Handbuch der Pflanzenphysiologie*, Vol. 16, Springer Verlag, Berlin/Göttingen/Heidelberg, 1961.
4 W. L. Butler, K. H. Norris, H. W. Siegelman and S. B. Hendricks, *Proc. Natl. Acad. Sci., U.S.*, 45 (1959) 1703.
5 S. B. Hendricks, H. A. Borthwick and R. J. Downs, *Proc. Natl. Acad. Sci., U.S.*, 42 (1956) 19.
6 S. B. Hendricks and H. A. Borthwick, *Proc. Natl. Acad. Sci., U.S.*, 45 (1959) 344.
7 S. B. Hendricks and H. A. Borthwick, *Botan. Gaz.*, 120 (1959) 187.
8 S. B. Hendricks, E. H. Toole, V. K. Toole and H. A. Borthwick, *Botan. Gaz.*, 121 (1959) 1.
9 H. Mohr, *Planta*, 49 (1957) 389.
10 H. Mohr, *Planta*, 53 (1959) 109.
11 H. Mohr, *Planta*, 53 (1959) 219.
12 H. Mohr, *Ergeb. Biol.*, 22 (1960) 67.
13 H. Mohr and I. Pichler, *Planta*, 55 (1960) 57.
14 H. Mohr and A. Noblé, *Planta*, 55 (1960) 327.
15 H. W. Siegelman and S. B. Hendricks, *Plant Physiol.*, 32 (1957) 393.
16 H. W. Siegelman and S. B. Hendricks, *Plant Physiol.*, 33 (1958) 185.
17 R. B. Withrow, W. H. Klein and V. Elstad, *Plant Physiol.*, 32 (1957) 453.

BIOLOGISCHE UHREN

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Uhren sind Instrumente zur Zeitmessung. Der Physiker unterscheidet unechte Zeitmessung, der ein einmaliger, gleichförmiger Naturvorgang zu Grunde liegt, von echter Zeitmessung, die auf gleichmässig wiederkehrenden (periodischen) Bewegungen beruht. Zu den ältesten unechten Uhren zählen die Wasser- und Sanduhren. Wird eine Sanduhr nach jedem Ablauf sofort wieder umgedreht, so entsteht bereits ein periodischer Vorgang, jedoch in der besonderen Form der Kippschwingung. Für echte Uhren benutzt die Technik meist das Prinzip der Pendelschwingung, die mit der Kreisbewegung eines rotierenden Körpers eng verwandt ist. Die Drehbewegung der Erde um ihre Achse hat zuerst dem Menschen die Möglichkeit geboten, periodische Vorgänge (Durchgang der Sonne oder eines Fixsternes durch den Meridian) zur Zeitmessung auszunutzen. Sie ist zugleich Ursache einer elementaren biologischen Uhr, die offenbar alle Lebewesen im Laufe der Evolution erworben haben.

Die 24-Stunden-Periodik ist jedoch durchaus nicht die einzige biologische Uhr. Höhere Organismen besitzen zahlreiche periodische Vorgänge sehr unterschiedlicher Frequenz, die alle in irgend einer Weise der Zeitmessung dienen könnten. Galilei soll seinen eigenen Puls gezählt haben, als er erstmals die Gleichförmigkeit einer Pendelschwingung messen wollte. Das wäre ein Fall bewusster Zeitmessung mit Hilfe biologischer Rhythmen, in denen u. a. Mach[1] Ursprünge der Chronometrie vermutet hat. Sehr viel zahlreicher jedoch sind solche Vorgänge in Organismen, in die unbewusste periodische Prozesse im Sinne von Uhren eingeschaltet sind. Eine kurze Übersicht über das Spektrum der hier zu berücksichtigenden Frequenzen mag zur spezielleren Behandlung des Themas überleiten.

I. ENDOGENE BIOLOGISCHE PERIODIZITÄTEN

Zeitmessungen können den Sinn haben, das „Richtige zur richtigen Zeit zu tun". Dabei kommt es unter Umständen nur darauf an, ein bestimmtes Ereignis in gleichen Zeitabständen zu wiederholen. Als Beispiel einer solchen Periodizität sei der Ovarialcyclus der Säugetiere genannt. Er gehört bei vielen Arten zur grossen Gruppe der rein endogenen biologischen Rhythmen, von denen die relativ hochfrequenten Potentialschwankungen des zentralen Nervensystems, insbesondere die Hirnaktionsströme (EEG), das kleinwellige Ende eines sehr breiten Spektrums bilden[2]. Die rein endogenen Rhythmen sind dadurch gekennzeichnet, dass sie dem Organismus nicht von aussen aufgezwungen sind und mit keinem periodischen Vorgang der Umwelt übereinstimmen. Ihre Bedeutung ist im allgemeinen auf Funktionszusammenhänge in

Organismus selbst beschränkt. Einige von ihnen mögen insofern als Uhren betrachtet werden, als sie dazu dienen, mehrere Vorgänge zu synchronisieren. Eine solche Synchronisation von Vorgängen einer bestimmten Frequenz innerhalb mehrerer Systeme oder Organe ist sicherer gewährleistet, wenn alle Systeme gleichlaufende Uhren besitzen und eine zentrale Mutteruhr lediglich den Gang der Nebenuhren kontrolliert.

Der Begriff der rein endogenen Periodizität lässt sich auf überindividuelle Einheiten ausdehnen. Der oben genannte Ovarialcyclus, der sich meist auch bei isoliert lebenden Weibchen spontan wiederholt, scheint bei gewissen Vogelarten nur dann periodisch weiterzulaufen, wenn die beiden Geschlechtspartner zusammenleben und sich mit ihren Funktionsabläufen gegenseitig synchronisieren[3]. Den nächsten Schritt bilden Periodizitäten, die nur innerhalb ganzer Populationen auftreten: Die Schwankungen der Populationsdichte, speziell bei manchen Säugetierarten, mit Periodendauern von 3 bis zu über 10 Jahren sind rein endogen In all diesen Fällen liegen die Ursachen der Periodik im biologischen System (in 1 Individuum, in 2 Individuen oder in der Population) und stehen in keinem ursächlichen Zusammenhang mit einer Umweltperiodik. Demgegenüber gibt es eine Klasse von Periodizitäten, die streng mit periodischen Vorgängen der Umwelt synchronisiert sind, darunter als die vier wichtigsten die Tages-Periodik, die Gezeiten-Periodik, die Lunar-Periodik und die Jahres-Periodik. Bei ihnen tritt der Charakter der Uhr wegen ihrer offensichtlichen Bedeutung für zeitrichtige Anpassung des Organismus an die periodische Umwelt deutlich hervor. Ein Beispiel für die Tagesperiodik bietet die Sonnenkompass-Orientierung vieler Vögel und Insekten. Für etliche dieser Zwecke könnte es genügen, wenn die biologischen Uhren nach jedem Umlauf von aussen neu angestossen werden müssten, also exogen erregt wären. Weit sicherer arbeiten jedoch auch hier wieder die echten Uhren, die selbsterregt (endogen) auch dann weiter laufen, wenn die steuernden Einflüsse der äusseren Uhr (der Umweltperiodik) künstlich ausgeschaltet werden.

Im breiten Spektrum endogener biologischer Rhythmen, das von Perioden mit der Dauer von Bruchteilen einer Sekunde bis zu vieljährigen Perioden reicht, bilden die vier mit der Umwelt synchronisierten Periodizitäten gewissermassen vier scharfe Spektrallinien. Allerdings ist von ihnen nur die 24-Stunden-Periodik mit Sicherheit als endogen erwiesen. Versuche von Hauenschild[4] am Polychaeten *Platynereis dumerilii* können im Sinne einer endogenen Lunarperiodik gedeutet werden; ähnliches mag für Gezeitenrhythmen gelten[5]. Bei der Jahresperiodik ist die Frage nicht endgültig entschieden, wenn auch eine Reihe von Befunden an Vögeln und Säugetieren die Hypothese einer endogenen Periodizität nahelegen[6,7]. Neuerdings hat Wolfson[8] an tropischen Vögeln, die über 18 Monate bei künstlicher Belichtung ohne jede jahreszeitliche Änderung gehalten wurden, zwei bis drei vollständige Brunstperioden beobachtet.

Eine an die Umwelt angepasste, endogene Periodizität ist nur sinnvoll, solange sie synchronisiert bleibt. Für diese Synchronisation sorgen bestimmte periodische Umweltgrössen, die als Zeitgeber[9,10] die Phase der biologischen Periodik bestimmen (Synchronizer[11]). Sie kontrollieren den Gang der biologischen Uhr, die ohne regelmässig wiederholte Korrektur nach kurzer Zeit hinter der Umwelt-Uhr zurückbleiben oder ihr vorauseilen würde. Art und Wirkweise solcher Zeitgeber für verschiedene Periodizitäten wurden an anderer Stelle ausführlich behandelt[12]. Bislang sind die Verhältnisse allerdings nur bei der 24-Stunden-Periodik genauer untersucht; sie sei deshalb im Folgenden als Musterbeispiel dargestellt.

2. DIE 24-STUNDEN-UHR

Man darf heute mit einiger Sicherheit annehmen, dass nahezu alle Organismen von den Einzellern bis zu den Primaten eine 24-Stunden-Periodik besitzen. Auch bei den Mehrzellern scheint sie in den einzelnen Zellen verankert zu sein; bei den höher entwickelten Organismen tritt hierzu möglichenfalls eine obergeordnete Zentraluhr. Der Gang der Uhr lässt sich am periodischen Ablauf einer oder mehrerer Funktionen des Organismus verfolgen. Für das Verständnis des Uhren-Mechanismus ist es weitgehend gleichgültig, welche Funktion hierfür gewählt wird. Vielfach bewährt ist die allgemeine lokomotorische Aktivität eines Tieres, die sich leicht und ohne Störungen des Objekts ununterbrochen messen lässt. Sie bildet die Grundlage eines Grossteils der hier zu erörternden Versuche an Vögeln und Nagern. Dabei ist im Auge zu behalten, dass die Messung anderer Organismen oder anderer Funktionen weitgehend zu denselben Ergebnissen führen würde (Vgl. Abb. 8).

Ob die unter natürlichen Bedingungen an einem Organismus beobachtete Tagesperiodik wirklich endogen ist und welche Faktoren der Umwelt für diesen Organismus als Zeitgeber wirksam sind, muss in jedem Fall mit besonderen Experimenten geprüft werden. Nach den bisher vorliegenden Ergebnissen ist der mit der Erddrehung verbundene Wechsel zwischen Licht und Dunkel der weitaus wichtigste Zeitgeber. Mit Hilfe künstlichen Belichtungswechsels wird deshalb auch im allgemeinen die Wirkweise von Zeitgebern untersucht. Im Folgenden sind drei Teilprobleme näher behandelt: (*1*) Die endogene Periodik unter konstanten Umweltbedingungen; (*2*) Die Wirkweise von Zeitgebern; (*3*) Vergleich der biologischen 24-Stunden-Periodik mit einer Modell-Pendelschwingung. Für eine Reihe weiterer Fragen sei auf die ausführliche Monographie von Bünning[13] verwiesen.

2.1. *Die Periodik unter konstanten Umgebungsbedingungen*

Im Laboratorium mit künstlichem Belichtungswechsel von 12 Std. Lichtzeit und 12 Std. Dunkelzeit (LD, 12 : 12) zeigt ein gekäfigter Buchfink eine deutlich perio-

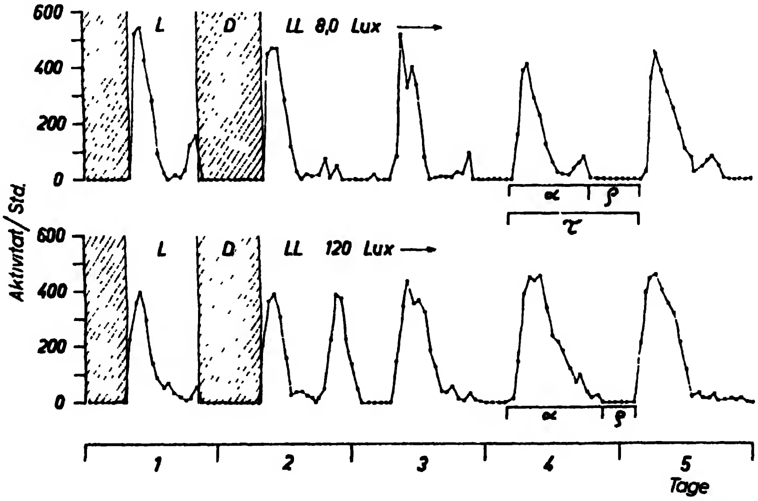

Abb. 1. Aktivitätsperiodik eines Buchfinken im künstlichen Wechsel zwischen 12 Std. Licht und 12 Std. Dunkel (L,D) und im Dauerlicht (LL) mit zwei Intensitäten. Die Periode τ gliedert sich in Aktivitätszeit α und Ruhezeit ρ.

dische Aktivität, mit einem Hauptmaximum zu Beginn und einem Nebenmaximum zu Ende der Lichtzeit. Diese Periodizität dauert an, wenn alle Umgebungsbedingungen konstant gehalten werden (Dauerlicht, dauerndes Futterangebot, konstante Temperatur usw.) (Abb. 1). Sie ist dann mit Sicherheit endogen, wenn die Periodendauer von 24 Std. abweicht. Nur in diesem Fall kann man sicher sein, dass keiner der zahlreichen tagesperiodischen Aussenfaktoren, die schwer oder nicht ausschliessbar sind (kosmische Strahlung, elektr. Potientiale usw.), als Ursache der Periodik in Frage kommt. Organismen mit einer von der Erddrehung abweichenden Spontanfrequenz (Lit.[12], free running period[14]) besitzen echte endogene Uhren, und für sie sind die synchronisierenden Aussenfaktoren echte Zeitgeber.

Jede Periode τ lässt sich ohne Zwang in zwei Abschnitte unterteilen (Abb. 1): Die Aktivitätszeit α und die Ruhezeit ϱ. Dies bedeutet nichts anderes, als dass die Werte der gemessenen Funktion während eines Teils der Periode über einen Schwellenwert ansteigen, während des anderen Teiles aber darunter bleiben. Im Falle der Aktivitätsmessungen hat diese Schwelle den Wert Null; bei anderen Funktionen, die — wie etwa die Körpertemperatur — stets oberhalb Null bleiben, tut jeder willkürliche Ordinatenwert (z.B. 37°) denselben Dienst. Bei gleichbleibendem τ sind Änderungen des $\alpha : \varrho$-Verhältnisses Ausdruck dafür, dass der mittlere Erregungspegel, um den das System schwingt, sich geändert hat (Lit.[15]; vgl. auch die beiden folgenden Abschnitte).

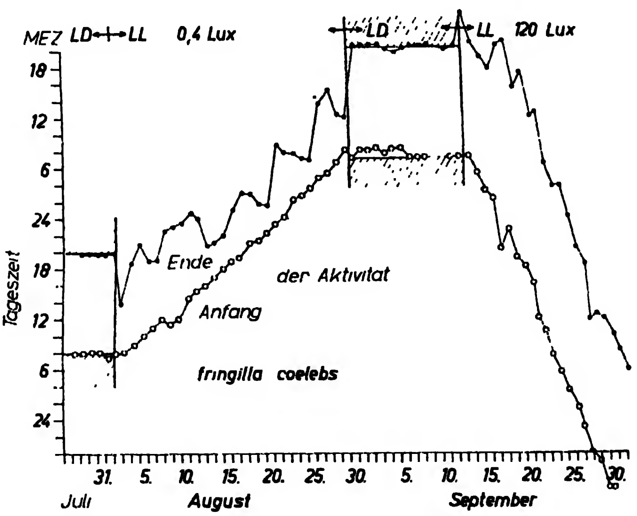

Abb. 2 Beginn ○ und Ende ● der Aktivität eines Buchfinken im künstlichen Belichtungswechsel (LD) und im Dauerlicht (LL), aufgetragen als Ordinatenwerte (in MEZ) gegen die Tagfolge als Abszisse. (aus Lit.[15]).

Die Genauigkeit, mit der ein Organismus seine Spontanfrequenz unter konstanten Umgebungsbedingungen einhält, lässt sich leicht zeigen, indem man in einem Diagramm den täglichen Beginn und das Ende der Aktivitätszeit als Ordinatenwerte gegen die Tagfolge als Abszisse aufträgt. (Abb. 2). Unter den Bedingungen künstlichen *Licht-Dunkel-Wechsels (LD)* beginnt und endet die Aktivität eines Buchfinken genau mit der Lichtzeit; die Verbindungslinien der beiden Punktfolgen laufen der Abszisse parallel. Im Dauerlicht (LL) von 0.4 Lux beginnt die Aktivität jeden Tag etwas später, in 120 Lux jeden Tag früher. Mit anderen Worten: Der Buchfink hat im

Dauerlicht eine Spontanfrequenz mit einer Periode von mehr als 24 Std. in 0.4 Lux und von weniger als 24 Std. in 120 Lux. Das Diagramm der Abb. 2 lehrt darüber hinaus dreierlei: *1)* Beim Übergang von LD zu LL setzt die Spontanfrequenz unmittelbar ein; beim Übergang von LL zu LD wird die Spontanfrequenz unmittelbar vom Zeitgeber eingefangen und mit 24 Std. synchronisiert; *2)* Die Aktivitätszeit ist in 0.4 Lux deutlich kleiner als in 120 Lux; *3)* Der Aktivitätsbeginn liefert ein genaueres Mass für die Frequenz als das Aktivitätsende. Die Länge einer Periode wird demnach zweckmässig vom Beginn einer Aktivitätszeit zum Beginn der nächsten gemessen.

Beobachtet man mehrere Vögel einzeln unter denselben Bedingungen und über längere Zeit, so zeigt sich, dass die gemessenen Spontanfrequenzen von Tier zu Tier etwas verschieden sind und dass sie sich bei ein und demselben Tier ohne ersichtlichen Grund ändern können. In Abb. 3 sind die Aktivitätsbeginne von 4 Buchfinken im

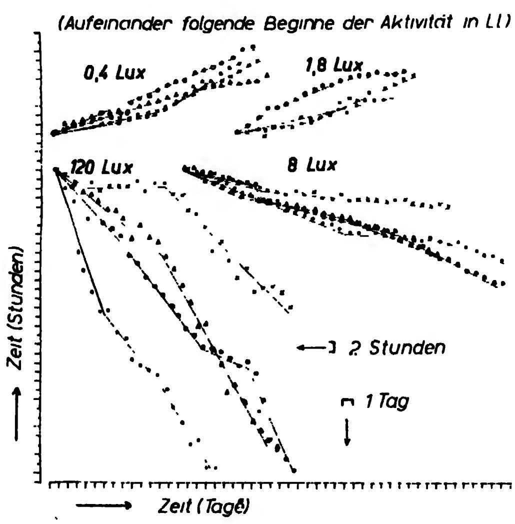

Abb. 3. Anfänge der Aktivitätszeiten von vier, einzel gemessenen Buchfinken im Dauerlicht viererlei Intensität. Beachte die unterschiedlichen Skalenwerte auf Ordinate und Abszisse[15].

Dauerlicht von 0.4, 1.8, 8 und 120 Lux eingezeichnet. Trotz der ersichtlichen intra- und interindividuellen Schwankungen lässt sich für jede der vier Beleuchtungsstärken eine mittlere Spontanfrequenz angeben; sie ist bei 0.4 Lux am kleinsten, bei 120 Lux am grössten. Diese Zunahme der Spontanfrequenz mit steigender Beleuchtungsstärke gilt für eine gewisse Gruppe von Organismen; zu ihr zählen die sogenannten lichtaktiven Tiere und offenbar auch einige Pflanzen. Bei dunkelaktiven Tieren wird dementgegen die Spontanfrequenz mit steigender Intensität des Dauerlichtes kleiner. Die Übersicht der Abb. 4 enthält alle bisher veröffentlichten Versuchsergebnisse, denen sich die Abhängigkeit der spontanen Periode von der Beleuchtungsstärke entnehmen lässt. Gegensinnig zu τ ändert sich meist α als Ausdruck des steigenden oder fallenden Erregungspegels. (Vgl. Abb. 7). Ohne einen solchen Zusammenhang zwischen Gesamterregung und Belichtung gäbe es keine Einteilung der Tiere in eine licht- und eine dunkelaktive Gruppe. Für die lichtaktiven Tiere entspricht dies der von Finsen[16] beschriebenen „lebenerweckenden" Wirkung des Lichts. Die Bedeutung des Pegels wird in den folgenden Abschnitten noch kurz zu erörtern sein.

2.2. *Wirkweise der Zeitgeber*

Ein periodischer Umweltvorgang kann nur dann Zeitgeber genannt werden, wenn der mit ihm synchronisierte Organismus eine endogene Periodizität besitzt. Das Studium des Zeitgebermechanismus setzt also die Verwendung von Organismen vor-

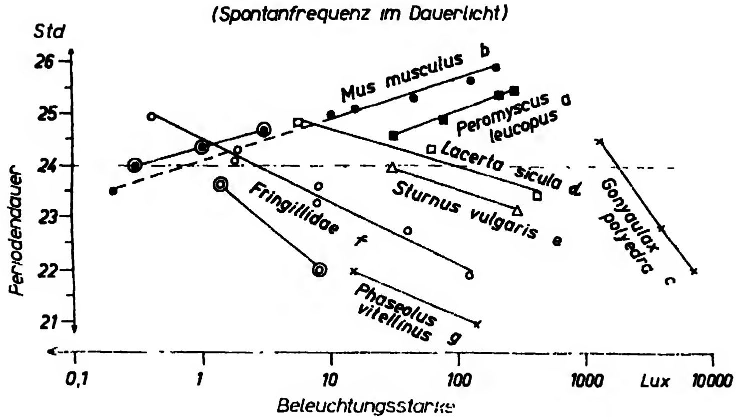

Abb. 4. Abhängigkeit der Periodendauer τ von der Beleuchtungsstärke im Dauerlicht für 7 verschiedene Organismen. Geschlossene Zeichen: dunkelaktive, offene Zeichen: lichtaktive Tiere. Doppelkreise: Zweite Versuchsserie an derselben Spezies. (Literatur bei[15]).

aus, bei denen eine Spontanfrequenz nachgewiesen ist. Ob für solch einen Organismus ein Umweltfaktor als Zeitgeber wirkt, lässt sich neben anderen mit 3 einfachen Experimenten prüfen:

(*1*) Einfangen der Spontanfrequenz. Ein Beispiel bietet die Abb. 2.

(*2*) Phasen-Verschiebung. Mit einem kräftigen Zeitgeber lässt sich die Phase der biologischen Periodik um jeden beliebigen Betrag gegen die Ortszeit verschieben. Die Abb. 5 zeigt das Verhalten eines Buchfinken im künstlichen Belichtungswechsel (LD, 12 : 12), dessen Phase um 12 Stunden verschoben wird, und zwar einmal durch Verdoppeln der Dunkelzeit (oben), das andere mal durch Verdoppeln der Lichtzeit (unten). In beiden Fällen ist der Vogel nach 4 bis 5 Perioden wieder synchronisiert; während der Synchronisationszeit ist die Amplitude gedämpft.

(*3*) Frequenz-Änderung. Künstlichen Frequenz-Änderungen eines wirksamen Zeitgebers folgt die biologische Periodik innerhalb gewisser Grenzen; die aufzwingbaren Frequenzen bilden den Ziehbereich. Ausserhalb des Ziehbereiches fällt der Organismus in eine Spontanfrequenz zurück. Beispiele solcher Experimente enthalten die Abb. 6 und 9.

Zeitgeber synchronisieren den Organismus mit einer bestimmten Frequenz dadurch, dass sie den Gang der endogenen Uhr genau um den Betrag korrigieren, den diese während einer Periode zu schnell oder zu langsam gelaufen ist. Korrigieren heisst: Die Phase der endogenen Periode um einen bestimmten Betrag nach vorn oder nach hinten zu verschieben. Dafür bestehen (ausser dem kurzfristigen Anhalten der Uhr) grundsätzlich zwei Möglichkeiten: (*A*) Jede Periodik lässt sich als Kreisbewegung rotierender Vektoren beschreiben. Während einer vollen Periode der Grundschwingung ist die Kreisfrequenz ω (= Winkelgeschwindigkeit des beschreibenden Vektors) nur bei einer reinen Sinusschwingung gleichförmig; bei jeder selbsterregten Schwingung ist der Augenblickswert der Kreisfrequenz während eines Teiles der Periode

Literatur S. 62

grösser, während eines anderen Teiles der Periode kleiner als die mittlere Kreisfrequenz. Phasenverschiebungen ($\Delta\varphi$) sind dadurch zu erreichen, dass die Kreisfrequenz während eines Teiles der Periode zusätzlich erhöht oder erniedrigt wird ($\Delta\varphi = \Delta\omega . t$). (*B*) Jede sprunghafte Änderung des Niveaus (Pegels), um das das System schwingt, führt zu einer unmittelbaren Phasenverschiebung.

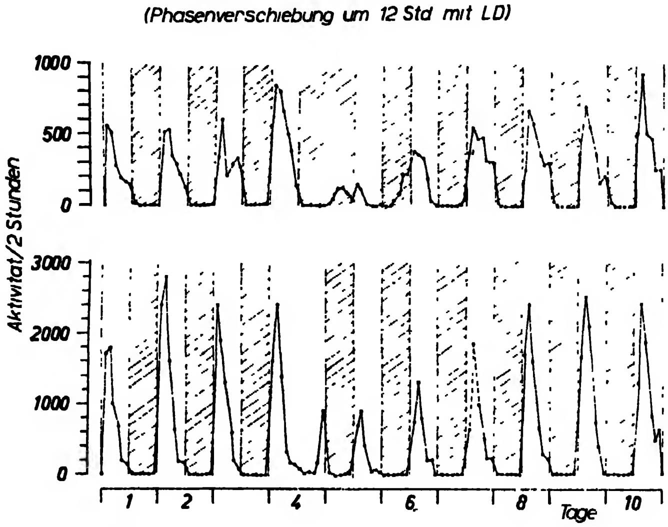

Abb. 5. Aktivität eines Buchfinken im künstlichen Belichtungswechsel (LD, 12 : 12). Plötzliche Phasenverschiebung des Zeitgebers um 12 Stunden, oben durch Verdoppeln der Dunkelzeit, unten durch Verdoppeln der Lichtzeit. (Nach Aschoff, *Mitt. Max-Planck-Ges.* 1959).

Es bleibt zu prüfen, welcher Parameter einer periodischen Umweltgrösse als Zeitgeber wirkt und über welchen Mechanismus er die Phase der biologischen Periodik korrigiert. Am künstlichen Licht-Dunkel-Wechsel lassen sich die beiden Möglichkeiten am leichtesten erörtern: (*a*) Wirksam sind die beiden Zustände, d.h. die Lichtzeit und die Dunkelzeit; (*b*) wirksam sind nur die Übergänge von einem Zustand zum andern, d.h. die Augenblicke „Licht-an" und „Licht-aus". Nach einem Vorschlag von Wever[17] spricht man im ersten Fall von einem Proportional-, im zweiten Fall von einem Differential-Effekt des Zeitgebers. Proportional-Wirkungen sind Reaktionen, die ein gleichbleibender Zustand des Zeitgebers im Organismus verursacht, sie dauern an, solange der Zustand unverändert bleibt. Differential-Wirkungen beruhen ausschliesslich auf Änderungen der Zeitgrösse. Die im vorigen Abschnitt erörterten Zusammenhänge legen den Gedanken nahe, dass der Proportional-Effekt eines LD-Zeitgebers in Änderungen der Kreisfrequenz des biologischen Systems besteht; diese hat bei lichtaktiven Tieren die Tendenz, in der Lichtzeit grösser, in der Dunkelzeit kleiner zu werden. Für dunkelaktive Tiere gilt das Gegenteil. Wenn die Belichtung gleichzeitig den Erregungspegel verändert, so ergibt sich beim Wechsel zwischen Licht- und Dunkelzeit zusätzlich ein Differentialeffekt. Theoretische Überlegungen machen es wahrscheinlich, dass Pegeländerungen zwangsläufig mit Änderungen der Kreisfrequenz verknüpft sind. Das würde bedeuten, dass Differential- und Proportional-Effekte mehr oder weniger immer zusammengehen. Die Frage, wie stark natürliche Zeitgeber proportional oder differential wirken, muss für jeden Zeitgeber und jeden Organismus besonders geprüft werden.

Sind Zeitgeber und Organismus miteinander synchronisiert, so besteht zwischen den beiden Perioden eine ganz bestimmte Phasenbeziehung. Jeweils nur eine solche Phasenwinkel-Differenz ist stabil; sie richtet sich nach der Art des Organismus und der des Zeitgebers. Die Abb. 6 bringt zwei Beispiele solcher Phasenbeziehungen, links

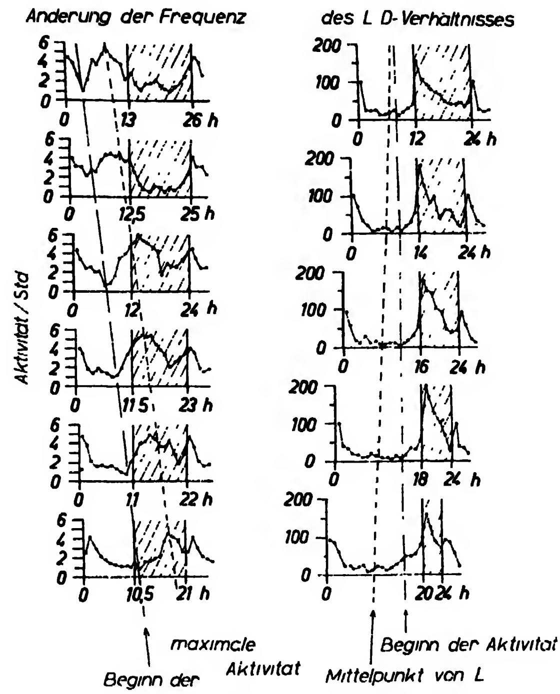

Abb. 6. Aktivität von Mäusen im künstlichen Belichtungswechsel, links verschiedener Frequenz, rechts verschiedenen L : D-Verhältnisses. Jede Kurve aus den Werten dreier Tiere über mehrere volle Perioden gemittelt. (Aus Lit.[15]).

für verschiedene Frequenzen eines Zeitgebers mit gleichbleibendem L : D-Verhältnis (50% L), rechts für gleichbleibende Frequenzen und verschiedene L : D-Verhältnisse. Versuchsobjekte sind in beiden Fällen Mäuse. Jedes einzelne Diagramm entspricht den Mittelwerten von drei Tieren und wenigstens 6 Perioden. Als Bezugspunkte der biologischen Periodik dienen die Minima der Aktivität (= Aktivitätsbeginn) oder die Maxima. Als Bezugspunkte des Zeitgebers könnten sowohl die Momente „Licht-an" und „Licht-aus" wie die Mittelpunkte der beiden Teilperioden herangezogen werden. Ein Blick auf die rechte Seite der Abb. 6 lehrt, dass bei gleichbleibender Zeitgeberfrequenz der Phasenwinkel des Organismus stets etwa dieselbe Differenz zu „Licht-aus" behält, wenn das Verhältnis von Licht-zu Dunkelzeit geändert wird; die Phasenwinkeldifferenz zwischen Mittelpunkt der Lichtzeit (oder Dunkelzeit) und Organismus ändert sich. Dies kann für ein Überwiegen der Proportional- über die Differential-Effekte sprechen. (Bei gleichstarker differentieller Steuerung durch „Licht-an" und „Licht-aus" sollte die biologische Phase an die Mittelpunkte der beiden Zeitgeber-Teilstrecken gebunden bleiben). Ein recht anderes Bild bietet die linke Hälfte der Abb. 6. Bei jeder Frequenz des Zeitgebers stellt der Organismus eine andere Phasenwinkeldifferenz ein, sowohl im Bezug auf die Mittelpunkte wie auf die Übergangsmomente des Zeitgebers. Bei niedriger Frequenz des Zeitgebers (26 Std. Perioden-

dauer) beginnt die Aktivität der Mäuse in der ersten Hälfte der Lichtzeit, die Tiere sind weitgehend lichtaktiv; bei hoher Zeitgeberfrequenz (21 Std. Periodendauer) ist der Aktivitätsbeginn ganz in die Dunkelzeit verschoben, die Mäuse sind überwiegend dunkelaktiv.

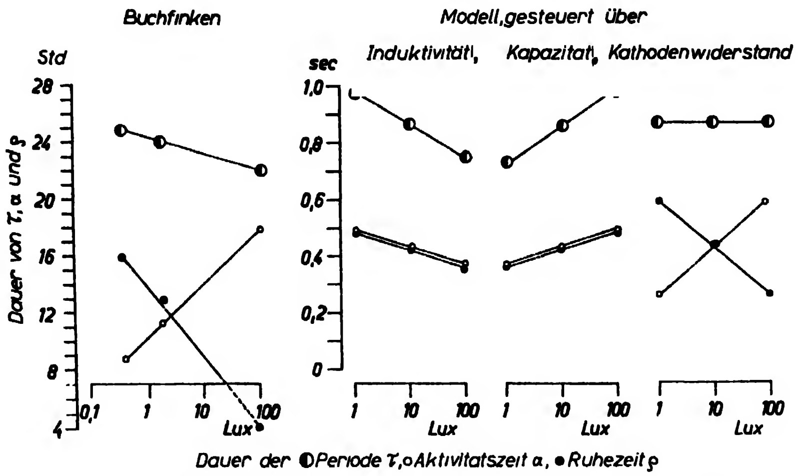

Abb. 7. Periodendauer τ, Aktivitätszeit α und Ruhezeit ρ als Funktion der Belichtungsstärke im Dauerlicht. Vergleich des Organismus (Mittelwerte für drei Buchfinken, ganz links) mit einem elektrischen Schwingkreis, bei dem entweder die Induktivität (links), die Kapazität (mitte) oder der Kathodenwiderstand (rechts) lichtempfindlich gemacht ist. (Modell nach Lit.[17]).

2.3. *Vergleich mit einer Pendelschwingung*

Im Gegensatz zu der oft vertretenen Hypothese, dass die Tagesperiodik einer Kippschwingung vergleichbar sei, hat Wever[17] an unserem Institut gezeigt, dass ein einfaches Pendelschwingungs-Modell die Mehrzahl aller am biologischen Objekt festgestellten Tatsachen zu beschreiben gestattet. Es handelt sich um einen rückgekoppelten elektrischen Schwingkreis mit einer Periodendauer von rund 1 sec. Wever hat abwechselnd drei entscheidende Parameter des Schwingkreises lichtempfindlich gemacht: Die Induktivität und die Kapazität, die beide die Frequenz der Schwingung bestimmen, und den Kathodenwiderstand, über den das mittlere Energieniveau (der Pegel) der Schwingung beeinflusst werden kann. Durch entsprechende Schaltung ist das Modell in allen drei Fällen so ausgelegt, dass bei Synchronisation mit periodischem Licht-Dunkel-Wechsel der Anodenstrom während der Lichtzeit sein Maximum und während der Dunkelzeit sein Minimum erreicht. Der Oszillator verhält sich also wie ein „lichtaktives Tier". Mit dem Modell lassen sich alle Versuche wiederholen, die im Vorhergehenden als Tierexperimente beschrieben sind. Genau so wie dort lässt sich prüfen, wie sich die Frequenz des Oszillators bei den drei verschiedenen Schaltungen mit der Belichtungsstärke (bei dauernder Belichtung) ändert und welche Phasenwinkeldifferenzen im Licht-Dunkel-Wechsel stabil sind. Auch lässt sich ebenso wie beim Organismus die ganze Periode τ in Aktivitätszeit α und Ruhezeit ρ trennen, indem der quantitativ registrierte Anodenstrom durch eine Schwelle in zwei Teile (oberhalb und unterhalb der Schwelle) zerlegt wird. Als Phasen-Bezugspunkte sind die Maxima jeder Schwingung gewählt.

Die Abb. 7 zeigt die Verhältnisse bei Dauerlicht. Für Vögel (linkes Diagramm) ist das Ergebnis bekannt: Frequenz und Aktivitätszeit werden mit steigender Belichtungs-

stärke grösser. Dem entspricht das induktiv gesteuerte Modell bezüglich der Frequenz, das über den Kathodenwiderstand gesteuerte Modell bezüglich der Aktivitätszeit. Das kapazitiv gesteuerte Modell widerspricht dem biologischen System in beiden Messgrössen. Aus diesem Versuch lässt sich bereits ableiten, dass ein Modell, bei dem sowohl Induktivität wie Kathodenwiderstand lichtempfindlich sind und das über beide gleichzeitig gesteuert wird, dem Verhalten des biologischen Systems am nächsten kommen muss. Diese Schlussfolgerung wird von den folgenden Versuchen bestätigt.

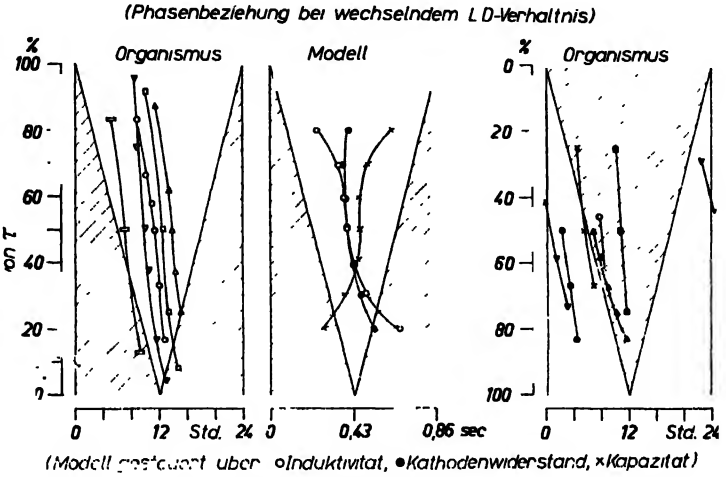

Abb. 8. Phasenbeziehung zwischen dem Zeitgeber (LD-Wechsel gleichbleibender Frequenz) und Organismen (links und rechts) oder einem elektrischen Schwingkreis (mitte) bei wechselndem L : D-Verhältnis. Links: *Oryzias latipes*, ▽ *Pilobolus sphaerosporus*, ○ *Kalanchoe blossfeldiana*, □ *Calendula arvensis*, △ *Pseudosmittia arenaria*. Rechts: ● ▼ ■ *Mus musculus*, < *Blatta orientalis*, ▲ *Rattus norv.*, ○ *Mesocricetus auratus*. Mitte: ○ Induktivität, ● Kathodenwiderstand, × Kapazität lichtempfindlich. (Literaturhinweise bei[15]; Modell nach[17]).

In Abb. 8 sind Versuche mit einem LD-Zeitgeber gleichbleibender Frequenz und wechselnden L : D-Verhältnisses wiedergegeben. Linkes und rechtes Diagramm enthalten die Ergebnisse verschiedener Autoren, die mit 9 verschiedenen Organismen gearbeitet haben. Links sind „lichtaktive" Organismen eingetragen, rechts „dunkelaktive". Jeder Punkt entspricht dem Mittelwert mehrerer Perioden und meist mehrerer Organismen; als Phasen-Bezugspunkt sind im allgemeinen die Maxima der jeweils untersuchten Funktion gewählt. In überraschender Übereinstimmung besagen die Ergebnisse fast aller Versuche dasselbe: Die Phase des biologischen Systems verschiebt sich (mit einer Ausnahme) nicht ganz parallel zu den Zeitpunkten „Licht-an" bzw. „Licht-aus", hält sich aber auch nicht parallel zu den Mittelpunkten einer der beiden Teilperioden des Zeitgebers. Ein solches Verhalten ist zu erwarten, wenn proportionale und differentiale Effekte zusammenwirken. Das (lichtaktive) Modell zeigt ähnliche Phasenbeziehungen zum Zeitgeber wie die lichtaktiven Organismen, wenn Induktivität und Kathodenwiderstand lichtempfindlich sind. Das kapazitiv gesteuerte Modell verhält sich gegensätzlich und entspricht somit keinem der bislang untersuchten Organismen.

Als drittes Beispiel ist in Abb. 9 der Ziehbereich von Mäusen (oberes Diagramm, vgl. die linke Hälfte der Abb. 6) mit denen des Modells verglichen (untere Hälfte der

Abb. 9). Der Ziehbereich des induktiv gesteuerten Modells wird von den beiden Spontanfrequenzen begrenzt (gestrichelte Linien), die dieses Modell bei maximaler (LL) bzw. bei minimaler Belichtungsstärke (DD) hat. Das über den Kathodenwiderstand gesteuerte Modell hat bei beiden Belichtungsstärken dieselbe Spontanfrequenz

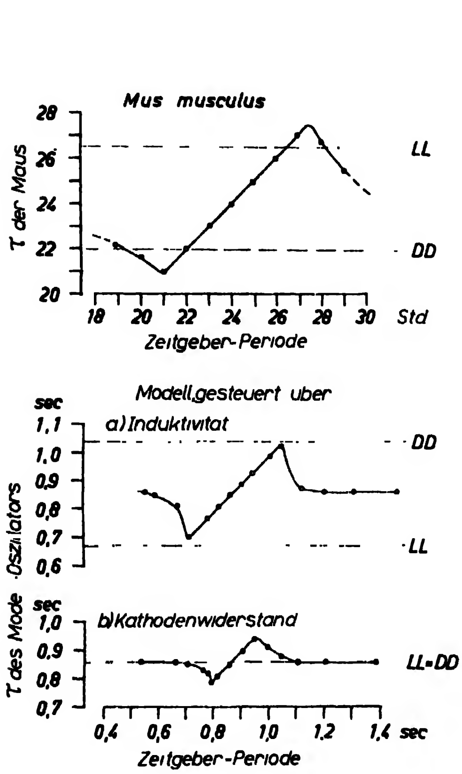

Abb. 9. Frequenzänderungen bei Mäusen (oben) und im elektrischen Schwingkreis (unten), erzwungen durch Frequenzänderungen eines LD-Zeitgebers (Strecke der Frequenzübereinstimmung zwischen beiden Schwimmungen = Ziehbereich). Im Modell ist einmal die Induktivität (*a*), das andere mal der Kathodenwiderstand (*b*) lichtempfindlich gemacht. Gestrichelt: Die Spontanfrequenzen mit denen die Systeme im Dauerlicht minimaler (DD) oder maximaler (LL) Beleuchtungsstärke schwingen. (Modell nach[17]).

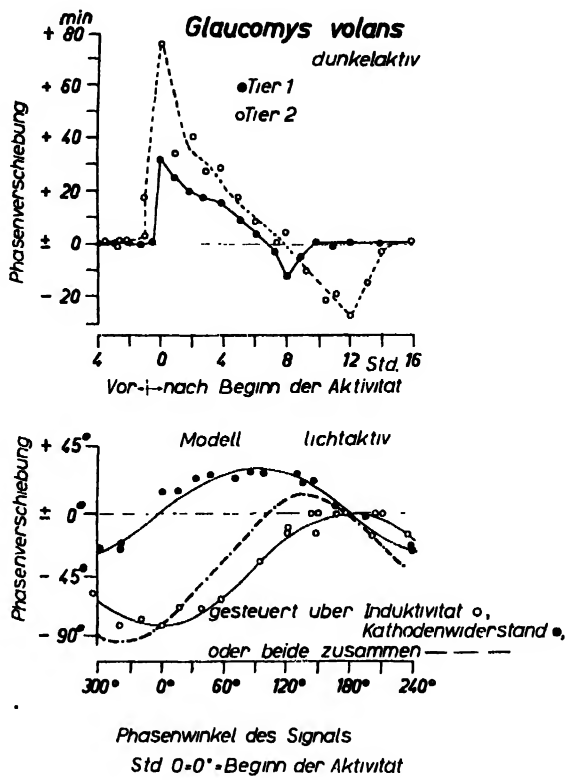

Abb. 10. Phasenverschiebungen, erzwungen durch kurzfristige Belichtung des im Dauerdunkel frei schwingenden Systems. Positive Werte: Verzögerungen, negative Werte: Beschleunigungen des Systems. Oben: Ergebnisse von De Coursey[30] am Flughörnchen. (2 Versuche). Unten: Elektrisches Modell, dessen Induktivität ○ oder Kathodenwiderstand ● oder beide zusammen lichtempfindlich sind (nach[17]). Abszisse: Zeitpunkt des Lichtreizes, gemessen vom Beginn der Aktivitätszeit.

(LL = DD); trotzdem gibt es auch hier einen Ziehbereich, der allerdings kleiner ist als beim induktiv gesteuerten Modell. Wenn der Ziehbereich von Mäusen über die Grenzen der bei ihnen im LL und im DD gefundenen Spontanfrequenzen hinausgeht, so lässt sich das wieder nur mit einem Modell beschreiben, das über Induktivität und Kathodenwiderstand zusammen gesteuert ist; es legt weiter die Annahme nahe, dass bei Mäusen proportionale und differentiale Zeitgebereffekte zusammenwirken. Bei 10 daraufhin geprüften Organismen entspricht die Phasenbeziehung in Experimenten mit Frequenzänderung weitgehend der, wie sie in der linken Hälfte der Abb. 6 für Mäuse gezeigt ist: Bei hoher Zeitgeberfrequenz ist die Phase gegenüber ihrer

Lage im 24-Std. Tag nach hinten, bei niedriger Frequenz nach vorne verschoben (Vergl. Lit.[15]).

Ein letztes Experiment sei an Hand von Abb. 10 besprochen. Weitaus der eleganteste Beweis für den Differentialeffekt eines LD Zeitgebers besteht darin, einen Organismus, der im LL oder im DD spontan schwingt, einem kurzen (= kurz in Bezug auf die ganze Periode) Licht-Blitz bzw. einem "Dunkel-Blitz" auszusetzen. Solche kurzfristigen Unterbrechungen der sonst konstanten Belichtungsverhältnisse verursachen unmittelbar eine Phasenverschiebung. Das Ausmass der Verschiebung richtet sich nach der Grösse des Differentials und nach der Phase, in der das System getroffen wird. Experimente dieser Art sind zuerst von Rawson[18] und von Bruce und Pittendrigh[19] ausgeführt worden. Die Abb. 10 enthält Ergebnisse, die De Coursey[20] an Flughörnchen gewonnen hat. Im DD angebotene Lichtreize von 10 Minuten Dauer bewirken bei diesen streng dunkelaktiven Tieren eine maximal positive Phasenverschiebung (Verzögerung) zu Beginn der Aktivitätszeit und eine geringere negative Verschiebung (Beschleunigung) etwa zu Ende der Aktivitätszeit; in der Ruhezeit scheint das Tier gegen Differentialeffekte mehr oder weniger unempfindlich zu sein. Im Gegensatz hierzu liegen die Phasenverschiebungen des induktiv gesteuerten Modells ausschliesslich auf einer Seite der Null-Linie. Bei Steuerung über den Kathodenwiderstand treten zwar positive und negative Phasenverschiebungen auf, sie liegen aber symmetrisch zur Null-Linie. Erst die Kombination beider Schaltungen (gestrichelte Kurve) bringt das richtige Ergebnis: Phasenverschiebung beiderseits der Null-Linie mit asymmetrischer Verteilung. (Die Kurve für das kombiniert gesteuerte Modell verläuft annähernd spiegelbildlich zu der der Flughörnchen, da diese dunkelaktiv sind, das Modell aber „lichtaktiv" geschaltet ist.)

Zusammengenommen zeigen die Versuche: Mit der Pendel-Schwingung eines elektrischen Schwingkreises lassen sich alle im Tierexperiment gefundenen Verhältnisse beschreiben, wenn Induktivität und Kathodenwiderstand lichtempfindlich sind und der Zeitgeber über beide das System steuert. Das Modell ermöglicht Voraussagen für den Ausgang bestimmter Experimente und legt die Hypothese nahe, dass der 24-Stunden-Periodik ein der Pendelschwingung verwandtes System zu Grunde liegt (also ein System mit zwei Energiereservoiren).

3. BEDEUTUNG DER UHR

Die 24-Stunden-Periodik, als die am besten untersuchte der vier mit der Umwelt synchronisierten Periodizitäten, ist zugleich ein vorzügliches Beispiel dafür, was solch eine Uhr für den Organismus bedeutet und was sie zu leisten vermag. Sie ist ein Musterfall von Anpassung, der zeigt, wie sich die Organismen im Laufe der Evolution darauf eingestellt haben, Gunst und Ungunst der Umwelt in ihrer periodischen Wiederkehr nicht einfach passiv hinzunehmen oder sich gegen sie so gut als möglich abzuschliessen (wie etwa mit Hilfe der Homoiothermie), sondern sich durch Entwicklung einer endogenen Uhr gleicher Umlaufzeit in jedem Augenblick bereits auf die Umstände des nächstfolgenden vorzubereiten. Auf diese Weise wurden die ursprünglichen „Ursachen" der Periodik durch nicht mehr ursächlich wirkende Zeitgeber abgelöst, die nur noch dafür sorgen, dass die erworbene endogene Periodik mit der der Umwelt Schritt hält, und die meist schon vor Eintritt der Umstände wirksam sind, die es zu meistern oder auszunützen gilt. Darüber hinaus ermöglicht die endogene

Literatur S. 62

Uhr die zeitliche Koordination bestimmter Einzelereignisse im Organismus mit solchen der Umwelt – z.B. das Aufsuchen des Futterplatzes zur richtigen Zeit — und die stete zeitgerechte Verrechnung etwa des Sonnenlaufes, wenn die Sonne als Kompass benutzt werden soll. Ein solcher Grad von Anpassung bedeutet notwendig eine Spezialisation, die ihre verwundbaren Punkte hat. Wird der in seiner natürlichen Umwelt mit dieser und in sich selbst voll synchronisierte Organismus in eine künstliche Umwelt gebracht, in der die verschiedenen Zeitgeber gegeneinander phasenverschoben sind oder in der sie fehlen, so geht auch die Synchronisation der Uhren im Organismus verloren. Die Folge sind Störungen des optimalen Funktionsablaufes[21] und echte Schädigungen[22]. So gilt für die Zeit eher noch mehr als für den Raum der Satz, dass der Organismus nicht ohne Gefahr die Umwelt verlässt, an die er angepasst ist.

Man hat zuweilen das Leben des Individuums mit einer Uhr verglichen, die mit seinem Tode still steht. Das gilt für die Tages- und Jahresuhr. Das ganze Leben jedoch ist nur der Pendelschlag einer Uhr mit weit grösserer Periodendauer. Die Folge der Generationen erst bildet wieder eine Periodizität, die als ein Instrument der Zeitmessung betrachtet sein mag. Es ist die Uhr, mit der die Evolution ihre Zeit misst; eine rein endogene Uhr, während deren Gang die Tages-Uhr sich entwickelte.

LITERATUR

1 E. Mach, *Erkenntnis und Irrtum*, 2. Aufl., Leipzig, 1906.
2 J. Aschoff, *Nova acta Leopoldina*, 21 (1959) 147.
3 D. S. Lehrman, *Trans. N. Y. Acad. Sci.*, 21 (1959) 682.
4 C. Hauenschild, *Naturwissenschaften*, 43 (1956) 361.
5 E. Naylor, *J. Exptl. Biol.*, 35 (1958) 602.
6 A. J. Marshall, *Cold Spring Harbor Symp. Quant. Biol.*, 25 (1960) 499.
7 J. Aschoff, *Studium generale*, 8 (1955) 42.
8 A. Wolfson, *Cold Spring Harbor Symp. Quant. Biol.*, 25(1960) 507.
9 J. Aschoff, *Naturwissenschaften*, 38 (1951) 506.
10 J. Aschoff, *Naturwissenschaften*, 41 (1954) 49.
11 Fr. M. Halberg, B. Visscher und J. J. Bittner, *Am. J. Physiol.*, 179 (1954) 229.
12 J. Aschoff, *Z. Tierpsychol.*, 15 (1958) 1.
13 E. Bünning, *Die physiologische Uhr*, Springer, Berlin/Göttingen, 1958.
14 C. S. Pittendrigh und V. G. Bruce, in Withrow (Editor), *Rhythmic and Synthetic Processes in Growth*, Princeton Univ. Press, 1957, p. 75.
15 J. Aschoff, *Cold Spring Harbor Symp. Quant. Biol.*, 25 (1960) 11.
16 N. R. Finsen, *Hospitalstidende*, (1895) 8.
17 R. Wever, *Cold Spring Harbor Symp. Quant. Biol.*, 25 (1960) 197.
18 K. Rawson, Ph.D. Thesis, Harvard University, 1956.
19 V. G. Bruce und C. S. Pittendrigh, *Am. Naturalist*, 92 (1958) 295.
20 P. J. de Coursey, *Ph.D. Thesis*, Univ. of Wisconsin, 1959.
21 F. W. Went, *Cold Spring Harbor Symp. Quant. Biol.*, 25 (1960) 221.
22 C. S. Pittendrigh, *Cold Spring Harbor Symp. Quant. Biol.*, 25 (1960) 159.

Symposium I

STRAHLUNGSKLIMA UND SEINE MESSUNG

Chairman: R. Schulze, Hamburg, Germany

Secretary: K. G. Hansen, Copenhagen, Denmark

ZUM STRAHLUNGSKLIMA DER ERDE

RUDOLF SCHULZE

Deutscher Wetterdienst, Meteorologisches Observatorium, Hamburg (Deutschland)

Zur Eröffnung des ersten Symposiums des Finsenkongresses 1960 schenkte mir Herr Madsen* den Erstdruck eines Buches von Finsen, in dem Finsen seine Messungen zum Strahlungsklima Kopenhagen bekannt gibt. Der Wunsch Finsens nach entsprechenden Angaben für die gesamte Erdoberfläche soll heute zu seinem hundertsten Geburtstag erfüllt werden.

1. BESCHRÄNKUNG AUF DEN STRAHLUNGSGEWINN DES ERDBODENS – THESE

Von der Leitung des Kongresses bin ich beauftragt, meine Betrachtungen auf den Strahlungsgewinn des Erdbodens (insbesondere auf die Globalstrahlung $S + H$, empfangen von der horizontalen Fläche) zu beschränken. Wir stellen für die Globalstrahlung folgende These auf:

Das Strahlungsklima jedes Ortes auf der Erde hängt in erster Linie ab von:

1. extraterrestrischer Bestrahlung,
2. Bewölkung,

von geringerer Bedeutung sind:

3. Streuung an den Molekülen der Atmosphäre,
4. Absorption im Wasserdampf der Atmosphäre,
5. Streuung und Absorption am Aerosol der Atmosphäre (Trübung).

Dies ist im folgenden zu beweisen.

2. BESTRAHLUNGSSTÄRKE UND TAGESSUMMEN FÜR WOLKENLOSE TAGE

Wir unterscheiden zwischen Bestrahlungsstärke (kW/m²) und Tagessumme (kWh/m² Tag), weil der Arzt, Biologe und Techniker nach der "Belastung" (Bestrahlungsstärke) fragt, der Klimatologe, Botaniker, Ozeanograph und Geophysiker nach der "Wirkung" (Tagessumme).

2.1 *"Ohne Atmosphäre"*

Denkt man sich die Erdoberfläche von jeder Atmosphäre und Bewölkung befreit, so ist für jeden Ort der Erde die Bestrahlungsstärke (S_0) leicht aus dem Produkt Solarkonstante ($I_0 = 1.39$ kW/m²) mal dem Sinus der Sonnenhöhe (h) zu berechnen.

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Literatur S. 75

Die Sonnenhöhe kann für jeden Ort der Erde für jeden Tag und für jede Tagesstunde aus astronomischen Tabellen entnommen werden. Die Werte von S_o für verschiedene Sonnenhöhen bringt Abb. 1 in der oberen Kurve und die Tabelle I.

Für die Tagessummen genügt es, die Werte von S_o mit der Sonnenscheindauer zu multiplizieren. Man wird hierbei die Entfernung der Erde von der Sonne im Laufe des

TABELLE I

BESTRAHLUNGSSTÄRKE (kW/m²) FÜR VERSCHIEDENE SONNENHÖHEN*

	S_o	$(S+H)_\sigma$	S_α 1 cm	S_α 2 cm	S_α 4 cm	$(S+H)_T$ $T = 2.75$	$(S+H)_\beta$ $\beta = 0.1$	$\frac{(S+H)_\beta}{S_o}$
90°	1.39	1.33	1.32	1.30	1.27		1.12	0.81
60°	1.20	1.14	1.13	1.11	1.08	0.97	0.95	0.79
30°	0.70	0.63	0.64	0.63	0.61	0.51	0.50	0.72
10°	0.24	0.21	0.21	0.21	0.21	0.13	0.13	0.54

* S_o: ohne Atmosphäre. $(S+H)_\sigma$: nach Streuung an den Molekülen (Deirmendjian und Sekera[2]). S_α: nach Absorption im Wasserdampf (Hoelper[4]). $(S+H)_T$: gemessen bei Trübungsfaktor $T = 2.75$ (Reitz[6]). $(S+H)_\beta$: berechnet für Trübungskoeffizient $\beta = 0.1$ (Hinzpeter[7]), gleich "Normalkurve". $(S+H)_\beta/S_o$: relative Bestrahlung.

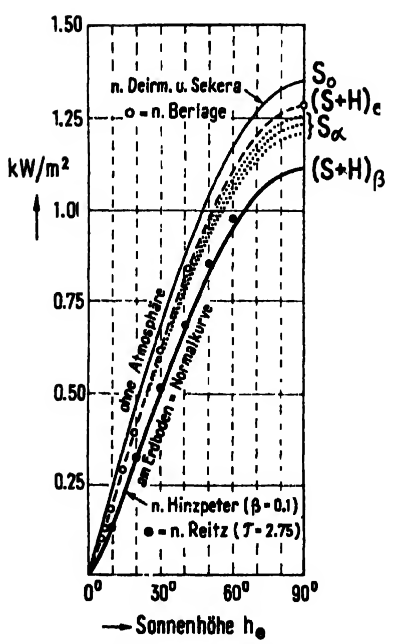

Abb. 1. Bestrahlungsstärke (kW/m²) der Globalstrahlung in Abhängigkeit von der Sonnenhöhe (S_o): ohne Atmosphäre. $(S+H)_\sigma$: nach Streuung an den Molekülen. S_α: n. Absorption im Wasserdampf (1,2 und 4 cm); $(S+H)_\beta$: Normalkurve (Hinzpeter[7]).

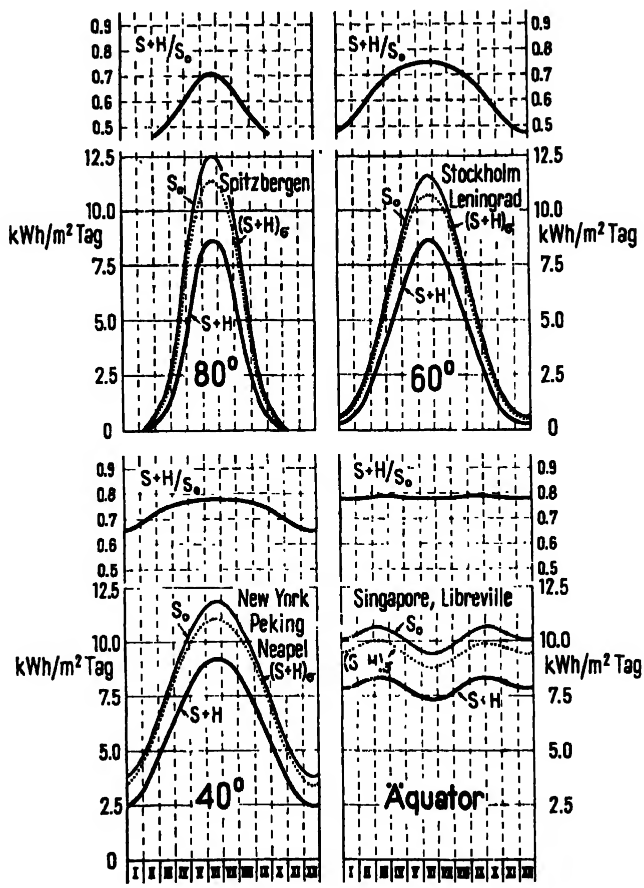

Abb. 2. Jahresgang der Tagessummen der Globalstrahlung (kWh/m²Tag) für verschiedene geographische Breiten. S_o: extraterrestr. Bestrahlung; $(S+H)$: an wolkenlosen Tagen zu erwartende Tagessummen: $(S+H)/S_o$; relative Bestrahlung.

Jahres mitberücksichtigen und eine genaue Integration durchführen. Diese Vorarbeit leistete Milankovitch[1], sein Ergebnis bringt für einige Orte die Abb. 2.

2.2. *Streuverlust an den Molekülen der Atmosphäre*

In der Atmosphäre werden etwa 10% der Sonnenstrahlung an den Molekülen der Atmosphäre gestreut. Etwa die Hälfte der gestreuten Strahlung erreicht als "Himmelsstrahlung" (H) den Erdboden. Die horizontale Fläche empfängt $(S+H)_\sigma$ nach der in Abb. 1 gestrichelt eingezeichneten Funktion, (Zahlenwerte s. Tabelle 1 nach Deirmendjian und Sekera[2]); die Reflexion am Erdboden wurde vernachlässigt. Für die Tagessumen berechnete Collmann[3] die entsprechenden Werte (s. Abb. 2 punktiert eingezeichnet).

2.3. *Absorptionsverlust am Wasserdampf der Atmosphäre*

Die Werte der Sonnenstrahlung nach Absorption im Wasserdampf der Atmosphäre bringt Tabelle I nach Hoelper[4]. Der Strahlungsverlust bei den einzelnen Wasserdampfdrucken unterscheidet sich nur um $\pm 2\%$, so dass man die Betrachtungen für das Strahlungsklima der Erde mit einer bestimmten Wasserdampfmenge — meist 1 cm H_2O gleich 7.3 mm Hg — durchführen kann.

Für die Tagessumme ergibt sich, dass die Summe des Streu- und Absorptionsverlustes von den Polen bis zum Äquator nahezu konstant ist, etwa 20%. Dies rührt daher, dass die in den Polargebieten flach einfallende Strahlung zwar stark gestreut, jedoch gering absorbiert wird, weil der Wasserdampfgehalt in den Polargebieten niedrig liegt. Am Äquator dagegen ist die Streuung niedrig, die Absorption hoch, weil die Sonnenstrahlung die Atmosphäre auf kurzem Wege durchsetzt, der Wasserdampfgehalt am Äquator jedoch hohe Werte annimmt (Schulze[5]).

2.4. *Strahlungsverlust am Aerosol — Trübung*

Der Staub und die sonstigen Verunreinigungen in der Atmosphäre, die wir mit Aerosol bezeichnen und die zur Trübung führen, schwächen die Sonnenstrahlung nochmals. Ihr Einfluss ist gering, weil ein grosser Teil der am Aerosol gestreuten Strahlung wieder durch Mehrfachstreuung den Erdboden erreicht. Die Summen aller Strahlungsverluste (Streuung an den Molekülen plus Absorption im Wasserdampf plus Schwächung am Aerosol) bringt Tabelle I und Abb. 1 als am Erdboden zu erwartende Globalstrahlung: $(S+H)_T$ sowie $(S+H)_\beta$. Die Übereinstimmung von Messung (Reitz[6]) und Berechnung (Hinzpeter[7]) ist überraschend gut. Der Trübungsfaktor $T = 2.75$ entspricht dem Trübungskoeffizienten $\beta = 0.1$; beide gelten als Mittelwerte für das flache Land. Im Hochgebirge werden niedrigere Trübungsfaktoren ($T = 1.9$) und über der Grossstadt höhere (im Mittel 3.75) beobachtet (Steinhauser[8]). Die anzubringenden Korrekturen für Hochgebirge und Grossstadt betragen rund 10%.

Die Abhängigkeit der Tagessummen vom Aerosol liegt ebenfalls bei $\pm$ 10%. Dies liegt wiederum daran, dass die Schwächung der direkten Sonnenstrahlung im Aerosol durch eine erhöhte Himmelsstrahlung nahezu aufgewogen wird. Aus den Werten der Bestrahlungsstärke der Tabelle I können die Tagessummen nach folgender Formel berechnet werden:

$$(S+H) = I_0 \sin h \, (0.46 + 0.54\, q^M)$$

Literatur S. 75

TABELLE II

TAGESSUMMEN DER GLOBALSTRAHLUNG AM ERDBODEN BEI WOLKENLOSEM HIMMEL IN kWh/m²TAG BERECHNET NACH DEN TABELLEN VON MILANKOVITCH[1]

	21. März	*6. Mai*	*22. Juni*	*8. Aug.*	*23. Sept.*	*8. Nov.*	*22. Dez.*	*4. Febr.*
Nordpol	—	5.65	8.77	5.60		—		—
80°	0.94	5.75	8.70	5.67	0.93	—		—
70°	2.20	6.22	8.58	6.16	2.17	0.14	—	0.14
60°	3.58	7.09	8.75	7.02	3.55	0.94	0.28	0.95
50°	4.92	7.85	9.07	7.78	4.86	2.17	1.21	2.18
40°	6.11	8.41	9.25	8.35	6.03	3.54	2.46	3.57
30°	7.11	8.71	9.15	8.62	6.95	4.92	3.92	4.97
20°	7.84	8.70	8.82	8.63	7.80	6.07	5.32	6.24
10°	8.29	8.49	8.19	8.34	8.19	7.29	6.68	7.35
Äquator	8.46	7.84	7.32	7.77	8.34	8.14	7.82	8.10
10°	8.29	7.02	6.25	6.95	8.19	8.75	8.75	8.89
20°	7.84	5.96	4.99	5.90	7.74	9.04	9.41	9.12
30°	7.10	4.74	3.67	4.67	7.02	9.01	9.79	9.26
40°	6.11	3.41	2.30	3.38	6.03	8.72	9.88	8.80
50°	4.92	2.09	1.12	2.08	4.86	8.14	9.70	8.22
60°	3.58	0.92	0.27	0.91	3.55	7.34	9.33	7.53
70°	2.20	0.13	—	0.13	2.17	6.45	9.15	6.51
80°	0.94	—	—	—	0.93	5.97	9.27	6.03
Südpol	—	—	—			5.87	9.35	5.93
	21. März	*6. Mai*	*22. Juni*	*8. Aug.*	*23. Sept.*	*8. Nov.*	*22. Dez.*	*4. Febr.*

worin $q = 0.7$ und M nahezu $1/\sin h$. Die Tabellen von Milankovitch[1] für $q = 1.0$ und $q = 0.7$ führen dann zu Tabelle II (Tagessummen der Globalstrahlung für die gesamte Erde in Abhängigkeit von der Jahreszeit).

2.5. *Einführung einer "Normalkurve" für die Globalstrahlung*

Zur Prüfung der Zuverlässigkeit der in Tab. I angegebenen $(S+H)_\beta$-Werte wurden eigene Strahlungsregistrierungen an wolkenlosen Tagen in Abhängigkeit von der Sonnenhöhe ausgemessen. Die Werte wurden bestätigt. Die maximale Streuung lag für Sonnenhöhen über 50° bei ± 3%; für 30°-Sonnenhöhe bei ± 6% und für 10°-Sonnenhöhe bei ± 20%. (Die Zunahme der Streuung bei niedrigen Sonnenhöhen ist auf instrumentelle Fehler zurückzuführen, u.a. weil unsere bisher benutzten Pyranometer oft für niedrige Sonnenhöhen eine gewisse Abweichung vom Cosinusgesetz zeigen).

Dieses Ergebnis sowie die gute Übereinstimmung mit den gemessenen Werten $(S+H)_T$ nach Reitz ermutigt uns, die $(S+H)_\beta$-Kurve der Abb. 1 als "Normalkurve" für unsere Betrachtungen des Strahlungsklimas der Erde anzusehen. Das Ergebnis bringt Abb. 2 für einige Städte (wolkenlose Tage): $S+H$-Tagessummen; ebenfalls Abb. 3.

2.6. *Einführung der "relativen Bestrahlung"*

Nach der These des Vorwortes sollte zwischen der zu erwartenden Globalstrahlung $(S+H)_\beta$ und der extraterrestrischen Bestrahlung (S_0) ein enger Zusammenhang bestehen; wir führen deshalb nach Fukui[9] die "relative Bestrahlung $(S+H)/S_0$ ein. Dieser Quotient hat den Vorteil, dass er frei ist von: Dimensionen, Winkelfunktionen, Entfernung Erde-Sonne. Die Werte für die Bestrahlungsstärke bringt Tabelle I, für die Tagessummen Tabelle III. Am Äquator erreichen danach nahezu 80% der extra-

terrestrischen Sonnenstrahlung den Erdboden, polwärts nimmt dieser Prozentsatz ab und ist abhängig von der Jahreszeit.

Alle bisherigen Überlegungen bezogen sich auf wolkenlose Tage.

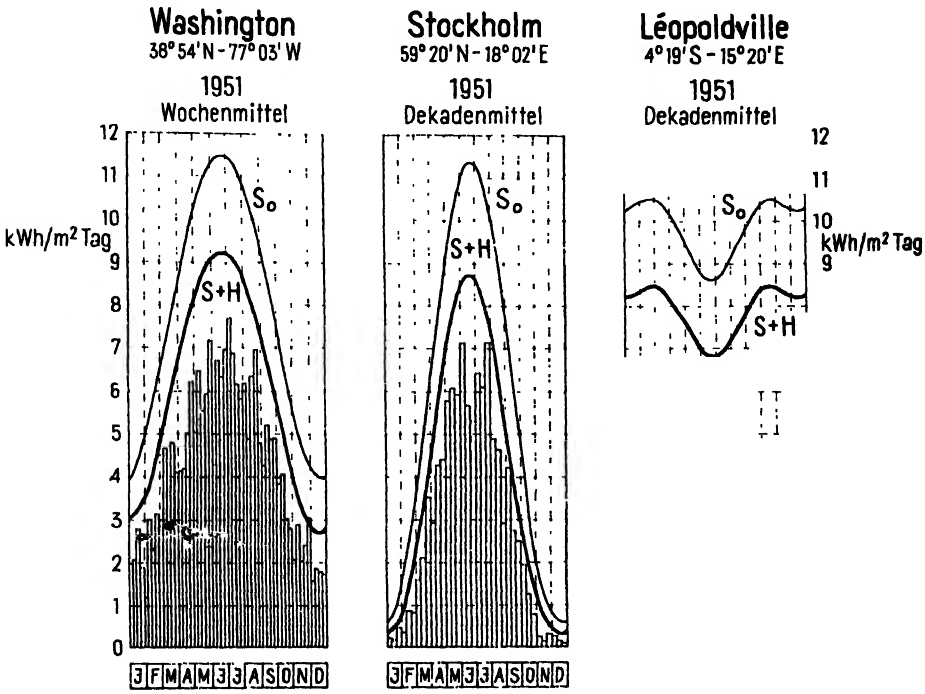

Abb. 3. Jahresgang der Tagessummen (kWh/m²Tag). S_0: ohne Atm.; $S+H$: am Erdboden für wolkenlose Tage; Kästchen Messwerte.

TABELLE III

$(S+H)/S_0$ AM ERDBODEN BEI WOLKENLOSEM HIMMEL, BERECHNET NACH DEN TABELLEN V MILANKOVITCH[1].

	21. März	*6. Mai*	*22. Jun.*	*8. Aug*	*23. Sept.*	*8. Nov*	*22. Dez.*	*4. Febr.*
Nordpol		0.61	0.68	0.61		—		—
80	0.50	0.62	0.69	0.62	0.50	-	—	—
70°	0.60	0.69	0.71	0.69	0.60	0.46	- -	0.46
60	0.66	0.73	0.75	0.73	0.67	0.54	0.47	0.55
50°	0.71	0.76	0.77	0.76	0.71	0.63	0.57	0.63
40°	0.74	0.78	0.78	0.77	0.75	0.69	0.65	0.69
30	0.76	0.78	0.78	0.78	0.76	0.73	0.70	0.73
20°	0.78	0.79	0.79	0.79	0.78	0.74	0.74	0.75
10°	0.79	0.79	0.78	0.78	0.79	0.77	0.76	0.77
Äquator	0.79	0.78	0.78	0.78	0.79	0.78	0.78	0.77
10°	0.79	0.77	0.76	0.77	0.79	0.78	0.78	0.79
20°	0.78	0.76	0.74	0.75	0.78	0.79	0.79	0.79
30°	0.77	0.73	0.70	0.73	0.77	0.78	0.78	0.79
40°	0.75	0.69	0.65	0.69	0.74	0.77	0.78	0.77
50°	0.72	0.63	0.57	0.63	0.71	0.76	0.77	0.76
60°	0.67	0.55	0.47	0.54	0.67	0.73	0.75	0.74
70°	0.60	0.46	-	0.46	0.60	0.69	0.71	0.69
80°	0.50	—	—	-	0.50	0.63	0.69	0.63
Südpol	—	—	—	—	—	0.61	0.68	0.61
	21. März	*6. Mai*	*22. Juni*	*8. Aug.*	*23. Sept.*	*8. Nov.*	*22. Dez.*	*4. Feb*

Literatur S. 75

3. EINFLUSS DER BEWÖLKUNG AUF DEN STRAHLUNGSGEWINN DES ERDBODENS

Als zweite wichtige Einflussgrösse für den Strahlungsgewinn des Erdbodens hatten wir in der anfänglichen These die Bewölkung genannt. Die Sonnenstrahlung wird an den Wolken z.T. reflektiert und in den Wolken z.T. absorbiert, so dass bei bedecktem Himmel oft nur 20% und weniger der Globalstrahlung den Erdboden erreichen.

Für die Tagessumme kann man Mittelwerte aus langjährigen Messreihen für den Bewölkungseinfluss errechnen, denen einige Genauigkeit (zumindest für Monatsmittel) beizumessen ist. Die Formeln gehen meist auf die ursprüngliche von Ångström[10] zurück. Vereinfacht wird das Problem, wenn man die relative Bestrahlung für den Zusammenhang Globalstrahlung und Bewölkung in die Rechnung einführt:

$$\frac{S+H}{S_0} \quad \frac{(S+H)_\beta}{S_0}(1 - 0.1B - 0.5B^2)$$

Die Werte $(S+H_\beta)/S_0$ sind für jeden Ort der Erde und für jeden Tag bekannt; B ist die Bewölkung in Zehnteln, wie sie laufend von den Wetterdiensten beobachtet wird.

Für bedeckten Himmel (10/10 Bewölkung) ergeben sich 28% der extraterrestrischen Sonnenstrahlung, bei $(S+H)_\beta/S_0 = 0.7$. Bei 6/10 Bewölkung verdoppeln sich die Prozente.

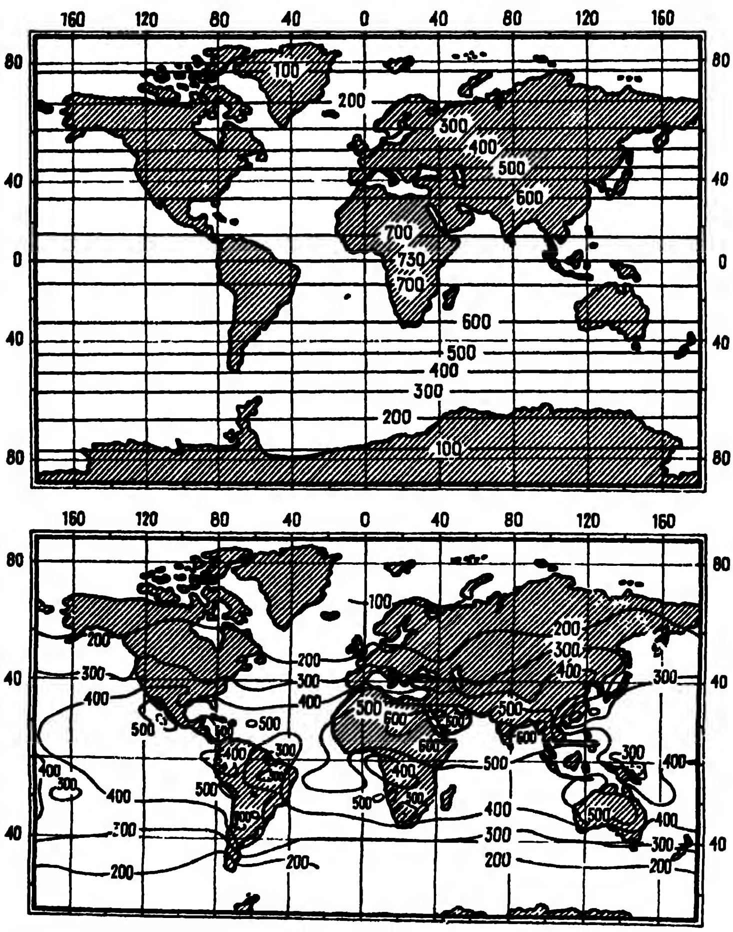

Abb. 4. Strahlungsklima der Erde in cal/cm²Tag für Monat März. Oben: für wolkenlose Tage (Verfasser); unten: mittlere Bewölkung (Black[11]). 100 cal/cm²Tag = 1.16 kWh/m²Tag.

Abb. 3 bringt Messergebnisse des Jahres 1951 für Stockholm, Washington und Léopoldville; sie sind zu Wochen- bzw. Dekadenmitteln der Tagessummen zusammengefasst (Kästchen). Zum Vergleich ist die zu erwartende Globalstrahlung für wolkenlose Tage ($S+H$) mit eingezeichnet. Die Differenz der Kästchen gegenüber ($S+H$) stellt den Einfluss der Bewölkung dar; die Bewölkung senkt die relative Bestrahlung im Jahresmittel auf 0.5; z.B. Stockholm: 0.49; Washington 0.53; Léopoldville: 0.44. Die Bewölkung beeinflusst also die Globalstrahlung wesentlich stärker als Streuung und Absorption in der Atmosphäre und im Aerosol, wie es nach der These zu beweisen war.

4. FOLGERUNGEN FÜR DAS STRAHLUNGSKLIMA DER ERDE

Die bisher gewonnenen Zahlenangaben genügen zur Beschreibung des Strahlungsklimas. Für wolkenlose Tage sind die Zahlenwerte der Tabelle II in Weltkarten einzuzeichnen (s. Abb. 4 und 5, oben). Für sämtliche Tage ist die mittlere Bewölkung mit

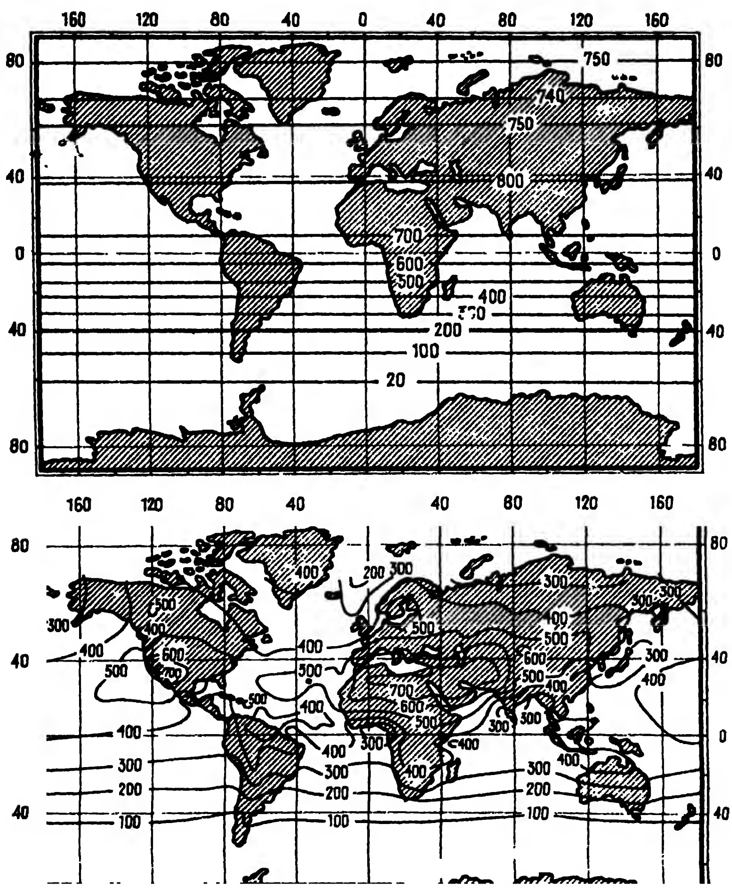

Abb. 5. Das gleiche wie Abb. 4, jedoch für Juni.

Hilfe der Bewölkungsformel zu berücksichtigen. Die unteren Karten der Abb. 4 und 5 wurden von Black[11] gezeichnet; sie stellen praktisch eine Multiplikation der oberen Karten mit dem prozentualen Strahlungsverlust durch die mittlere Bewölkung dar.

Literatur S. 75

Ihre Zuverlässigkeit hängt selbstverständlich von der Genauigkeit der Klimadaten für die mittlere Bewölkung ab. Es ist deshalb oft vorgeschlagen worden, statt der Bewölkungsformel die sehr gut bekannten Zusammenhänge Sonnenscheindauer-Globalstrahlung als Mass für den Einfluss der Bewölkung zu benutzen. Leider liegen jedoch zur Zeit keine weltweiten Klimaangaben über die Sonnenscheindauer vor.

Besonders aufschlussreich sind Weltkarten für die relative Bestrahlung, die hier aus Platzmangel nicht gezeigt werden können. Für den Monat März läuft $(S+H)/S_0 = 0.3$ parallel zum Breitenkreis 80°Nord; im Süden 0.4 parallel zu 40°Süd. Die Wüstengebiete heben sich durch Werte über 0.7 hervor. Über dem Weltmeere ergibt sich 0.4 ± 0.1.

5. ULTRAVIOLETTSTRAHLUNG

Ähnliche Überlegungen wie für die Globalstrahlung kann man auch für die Ultraviolettstrahlung ($0.29\,\mu$–$0.4\,\mu$) anstellen. Das besondere Merkmal der Ultraviolettstrahlung gegenüber der Globalstrahlung ist ihre hohe Streuung an den Molekülen der Atmosphäre, die nach kürzeren Wellenlängen nach dem Rayleighschen Gesetz umgekehrt zur 4. Potenz zu ansteigt. Dies hat zur Folge, dass die aus dem Himmelsgewölbe zum Erdboden kommende U.V.-B-Strahlung meist 10 bis 20 mal stärker ist als die direkte Sonnenstrahlung. Das Messverfahren muss also die U.V.-Strahlung aus dem gesamten Halbraum 2π (Himmelsgewölbe) empfangen.

Das Meteorologische Observatorium Hamburg des Deutschen Wetterdienstes hält

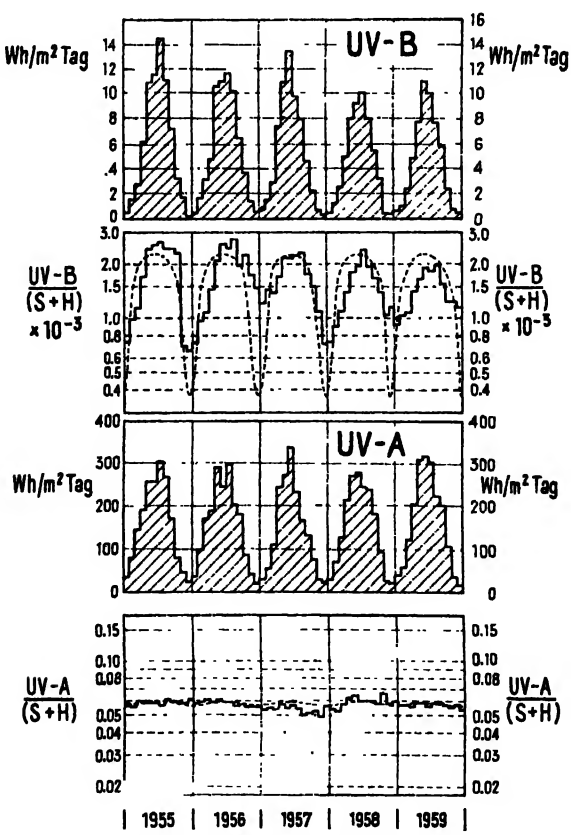

Abb. 6. Monatsmittel der Tagessummen der Ultraviolettstrahlung, gemessen vom Deutschen Wetterdienst in Hamburg (1955–1959).

hierfür die Larché-Kugel für geeignet (Fleischer[12]). Weitere Messverfahren werden in den folgenden Referaten bekanntgegeben. Die Abb. 6 bringt die Monatsmittel für die Jahre 1955–1959, wie sie vom Deutschen Wetterdienst im Meteorologischen Observatorium Hamburg gewonnen wurden. Die obere Darstellung gilt für das U.V.-B (kleiner als 0.32 μ); der dritte Kurvenzug von oben bringt Werte für das U.V.-A

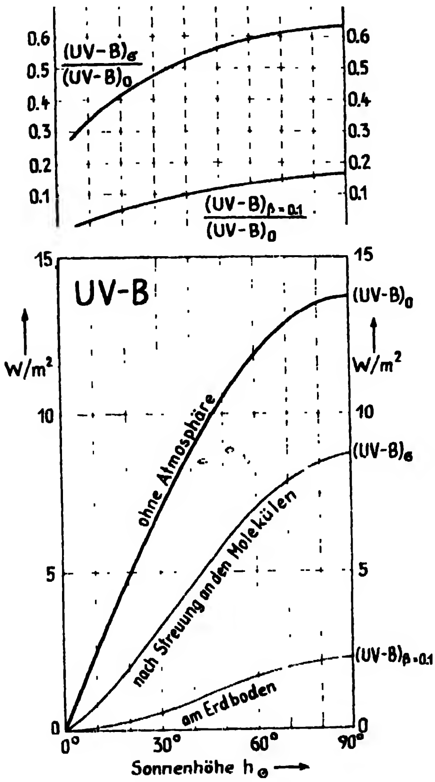

Abb. 7. Bestrahlungsstärke des U.V.-B. (U.V.-B)$_0$: ohne Atm. (Nicolet[13]); (U.V.-B)$_\sigma$: n. Streuung and den Molekülen[2]); (U.V.-B)$_{\beta=0.1}$: Normalkurve des U.V.-B; Trübungskoeffizient β = 0.1 (Hinzpeter[7]).

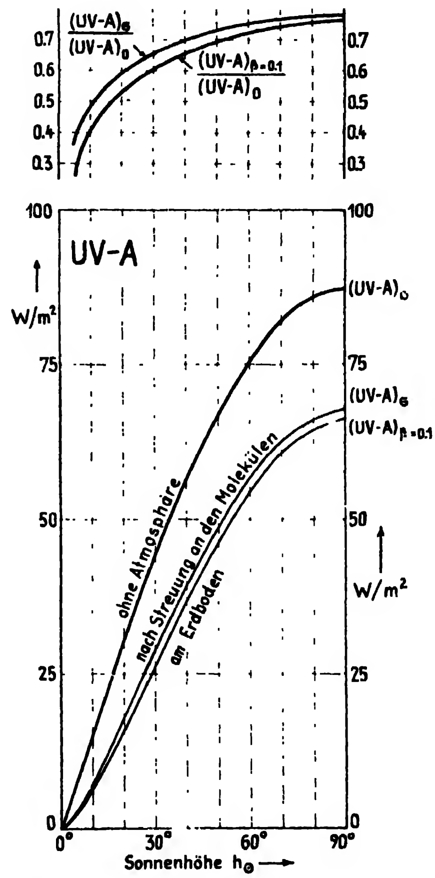

Abb. 8. Bestrahlungsstärke des U.V.-A. Bezeichnungen wie in Abb. 7.

TABELLE IV

BESTRAHLUNGSSTÄRKE DER U.V.-B SOWIE DER U.V.-A STRAHLUNG (W/m²) FÜR VERSCHIEDENE SONNENHÖHEN*

	$(U.V.\text{-}B)_0$	$(U.V.\text{-}B)_\sigma$	$(U.V.\text{-}B)_\beta$	$\frac{(U.V.\text{-}B)_\beta}{(U.V.\text{-}B)_0}$	$(U.V.\text{-}A)_0$	$(U.V.\text{-}A)_\sigma$	$(U.V.\text{-}A)_\beta$	$\frac{(U.V.\text{-}A)_\beta}{(U.V.\text{-}A)_0}$
90°	13.8	8.8	2.3	0.16	87	68	66	0.76
60°	11.9	7.0	1.7	0.14	76	57	54	0.72
30°	6.9	3.3	0.54	0.08	44	29	26	0.60
10°	2.4	0.8	0.05	0.02	15	7.4	6.1	0.40

* (U.V.-B)$_0$: ohne Atmosphäre (Nicolet[13]). (U.V.-B)$_\sigma$ n. Streuung an den Molekülen[2]. (U.V.-B)$_\beta$ berechnet für Trübungskoeffizient β = 0.1 (Hinzpeter[7]). Das Gleiche für U.V.-A.

Literatur S. 75

($0.32\ \mu$–$0.4\ \mu$). Die in Abb. 6 zusätzlich noch eingetragenen Quotienten geben das Verhältnis zur Globalstrahlung an.

5.1. *U.V. an wolkenlosen Tagen*

Die Bestrahlungsstärken für U.V.-B sowie für U.V.-A bringen Abb. 7 und 8 sowie Tabelle IV, analog zur Globalstrahlung für die extraterrestrische Strahlung $(U.V.)_o$, Nicolet[13], nach Streuung an den Molekülen $(U.V.)_\sigma$ sowie nach Streuung und Absorption in der Atmosphäre und im Aerosol $(U.V.)_\beta$, Hinzpeter[7]. Die Werte der relativen U.V.-Bestrahlung $(U.V.)_\beta/(U.V.)_o$ (ebenfalls Tabelle IV) weisen auf den Unterschied der starken Schwächung des U.V.-B gegenüber der wesentlich geringeren des U.V.-A hin, wie es auch die Abb. 7 und 8 deutlich zeigen. Nach den Berechnungen sollte der Einfluss des Gebirges bei etwa 15% Zuwachs pro 1000 Meter Höhe für U.V.-B und etwa 4% pro 1000 Meter Höhe für U.V.-A liegen. Dies konnte vom Verfasser[5] durch physikalische wie auch biologische Versuche bestätigt werden. Die U.V.-B-Bestrahlungsstärke ist für gleiche Sonnenhöhen auch fast unabhängig von der geographischen Breite. Die geringere Ozonmächtigkeit in den Tropen wird durch den höheren tropischen Dunst ausgeglichen (Büttner[14]). Im Herbst liegt die Bestrahlungsstärke höher als im Frühjahr (n. Götz). Der Dunst über den Grossstädten verringert das U.V.-B um etwa 10%. Mit 1000 m Höhe über NN steigt das U.V.-B in den Tropen um 20 bis 25% (nach Büttner[14]) an; weitere Einzelheiten s. Verfasser[5]. Die U.V.-A Bestrahlungsstärke zeigt diese Abhängigkeiten in weit geringerem Masse, weil sie in der Atmosphäre kaum Absorption erleidet — im Gegensatz zum U.V.-B.

Die Tagessummen des U.V.-B für wolkenlose Tage können zur Zeit noch nicht angegeben werden, weil die Rechentabellen fehlen. Die Tagessummen des U.V.-A für wolkenlose Tage dagegen lassen sich nach der Formel von Berlage[15] und den Tabellen von Milankovitch[1] für einen Transmissionsfaktor T = 0.6 leicht als Anhalt berechnen,

$$U.V.\text{-}A = 0.5\ (U.V.\text{-}A)_o \sin h\ (1 + 0.7^{1/\sin h}).$$

Messungen des Verfassers an der See und im Hochgebirge bestätigen sie.

5.2 *U.V.-Strahlung an bewölkten Tagen*

Als Beispiel für die Tagessummen am bewölkten Tagen des U.V.-B sowie des U.V.-A kann die Abb. 6 dienen; die Tabelle V bringt im Auszug deren Zahlenwerte für einige Monate. Besonders aufschlussreich sind die Verhältniszahlen U.V.-B : U.V.-A: $S+H$, die durch die Theorie für die Bestrahlungsstärken bestätigt werden (für 60°-Sonnenhöhe: 1 : 32 : 540 und für 30°-Sonnenhöhe: 1 : 48 : 910). Die Unterschiede

TABELLE V

MONATSMITTEL DER TAGESSUMMEN DER U.V.-B SOWIE DER U.V.-A-STRAHLUNG (kWh/m²Tag) GEMESSEN IN HAMBURG[16], 1955-1959.

	März	*Juni*	*Sept.*	*Dez.*
U.V.-B (kWh/m²Tag)	0.0028	0.0121	0.0061	0.0004
U.V.-A (kWh/m²Tag)	0.127	0.289	0.172	0.020
$S+H$ (kWh/m²Tag)	2.280	5.090	3.125	0.381
U.V.-B: U.V.-A: $S+H$	1:46:830	1:24:420	1:28:510	1:54:1030

beruhen auf dem Einfluss der Bewölkung. Die Werte für die Bestrahlungsstärke gelten für wolkenlose Tage unabhängig von der geographischen Breite; die Werte für die Tagessummen sind Mittelwerte unter Einschluss der Bewölkung über Hamburg.

LITERATUR

1 M. Milankovitch, *Handbuch d. Klimatol.*, I A, 1930, S. 14 und 62.
2 D. Deirmendjian und Z. Sekera, *Tellus*, 6 (1954) 382.
3 W. Collmann, *Ann. Meteorol.*, 8 (1958) 179; *Ber. deutsch. Wetterdienstes*, 42, VI (1958).
4 O. Hoelper, *Meteorol. Z.*, 54 (1937) 459.
5 R. Schulze, *Arch. Meteorol. Geophys. u. Bioklimatol.*, B 9 1958 254; *Strahlentherapie*, 111 (1960) 392; *Ann. Meteorol.*, I (1948) 12.
6 G. Reitz, *Gerlands Beitr. Geophys.*, 55 (1939) 289.
7 H. Hinzpeter, *Z. Meteorol.*, 9 (1955) 308; 10 (1956) 100; 11 (1957), 1.
8 F. Steinhauser, *Gerlands Beitr. Geophys.*, 42 (1934) 110.
9 Fukui, *Geophys. Mag. Tokio*, 23 (1952) 429.
10 A. Ångström, *Quart. J. Roy. Meteorol. Soc.*, 50 (1924) 121.
11 N. J. Black, *Arch. Meteorol. Geophys. u. Bioklimatol.*, B 7 (1956) 165.
12 R. Fleischer, *Geofis. pura e. apll.*, 26 (1953) 172.
13 M. Nicolet, *Arch. Meteorol. Geophys. Bioklimatol.*, B, III (1951) 209.
14 K. Büttner, *Physik. Bioklimatol.*, Leipzig, 1938; H. Pfleiderer und K. Büttner, *Bioklim.* im *Lehrb. f. Bäder und Klimaheilkunde*, 1940, S. 609.
15 H. P. Berlage, *Meteorol. Z.*, 45 (1928) 174.
16 Deutscher Wetterdienst, *Beilage zum Medizin-Met. Ber.* (*Strahlungswerte* 1954–1959).

THE INFLUENCE OF THE ALPS ON THE RADIATION CLIMATE

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What influence do the Alps have on the radiation climate? Two groups of factors are of decisive importance:

I. The shape of the terrain and the direction in which it slopes.

II. The alterations in weather brought about by the Alpine barrier.

The influence of these factors is very different, the first being static and the second largely dynamic in its effect.

I. THE SHAPE OF THE TERRAIN AND ITS DIRECTION OF SLOPE

How does the influence of the terrain shape and aspect on the radiation climate make itself felt?

Except on the actual peaks the path of the sun is shortened for all other places in the Alps to a more or less considerable degree. The effect of this on all elements of the radiation process is extremely various. It is particularly marked where any summation of amounts of daylight is involved.

Let us consider in this connection the following elements of radiation:

1. The duration of sunshine.
2. Direct solar radiation.
3. Radiation of sun + sky.
4. Diffuse radiation of the sky.
5. Circumglobal radiation.

Duration of sunshine

The shortening of the diurnal orbit is limited generally to the early and late hours where the area is relatively open, but it may extend to other parts of the orbit where the terrain is particularly enclosed. There are an almost unlimited number of possibilities, and it would be a hopeless undertaking, for instance, to try and make a map of the Alps showing the potential duration of sunshine (= effective possible duration of sunshine). This can be shown from two examples on the basis of measurements that have actually been made.

Example 1 — (Magadino plain at the southern foot of the Alps). We have here a plain 34 km^2 in area, some 200 m above sea-level, with its longitudinal axis running roughly east-west. The plain is surrounded by mountains which reach a height of more than

2000 m (Fig. 1). In order to map the potential duration of sunshine, the line of the horizon had to be fixed at more than 70 points by means of theodolites or instruments capable of recording the sun's path. Fig. 2, in which the isohels for the shortest and longest day are given, strikingly indicates how complicated the sunshine conditions are even in the case of such relatively simple topography[1].

Fig. 1. The plain of Magadino on the southern foot of the Alps.

Example 2 · (Baye de Montreux on the Lake of Geneva). This example is much more complicated because it involves not a plain but an area of complexly articulated slopes. The line of the horizon had to be fixed at 149 points in an area of 14 km^2. Fig. 3 shows only a section of this area. Certain simplifications were necessary here in order to make any record of the isohels.

How, then, does this shortening of the sun's path affect radiation? This seems to be an easy enough question as far as the duration of sunshine is concerned. Yet it would be a great mistake to assume, for instance, that one could calculate the real monthly sums of sunshine duration simply by multiplying the mean values of relative sunshine duration for a representative station ' v the potential sunshine duration for any station where orbit measurements have been made. For account must also be taken of the fact that the relative sunshine duration follows a marked daily course, having small values in the early and late hours and high values during the midday period (Fig. 4). This means that the shortening of the sun's orbit as a result of elevations of the horizon does not have as strong an effect as it would if there were no such variation in values throughout the day[2].

References p. 91

One thing at least is clear. If the duration of sunshine is to be an expression of the cloud and radiation conditions, then for the Alpine region two kinds of data must always be known: the actual duration as measured and the potential duration as determined by the line of the horizon. Otherwise an entirely false picture will emerge, as one cannot determine how the values of sunshine duration have been arrived at.

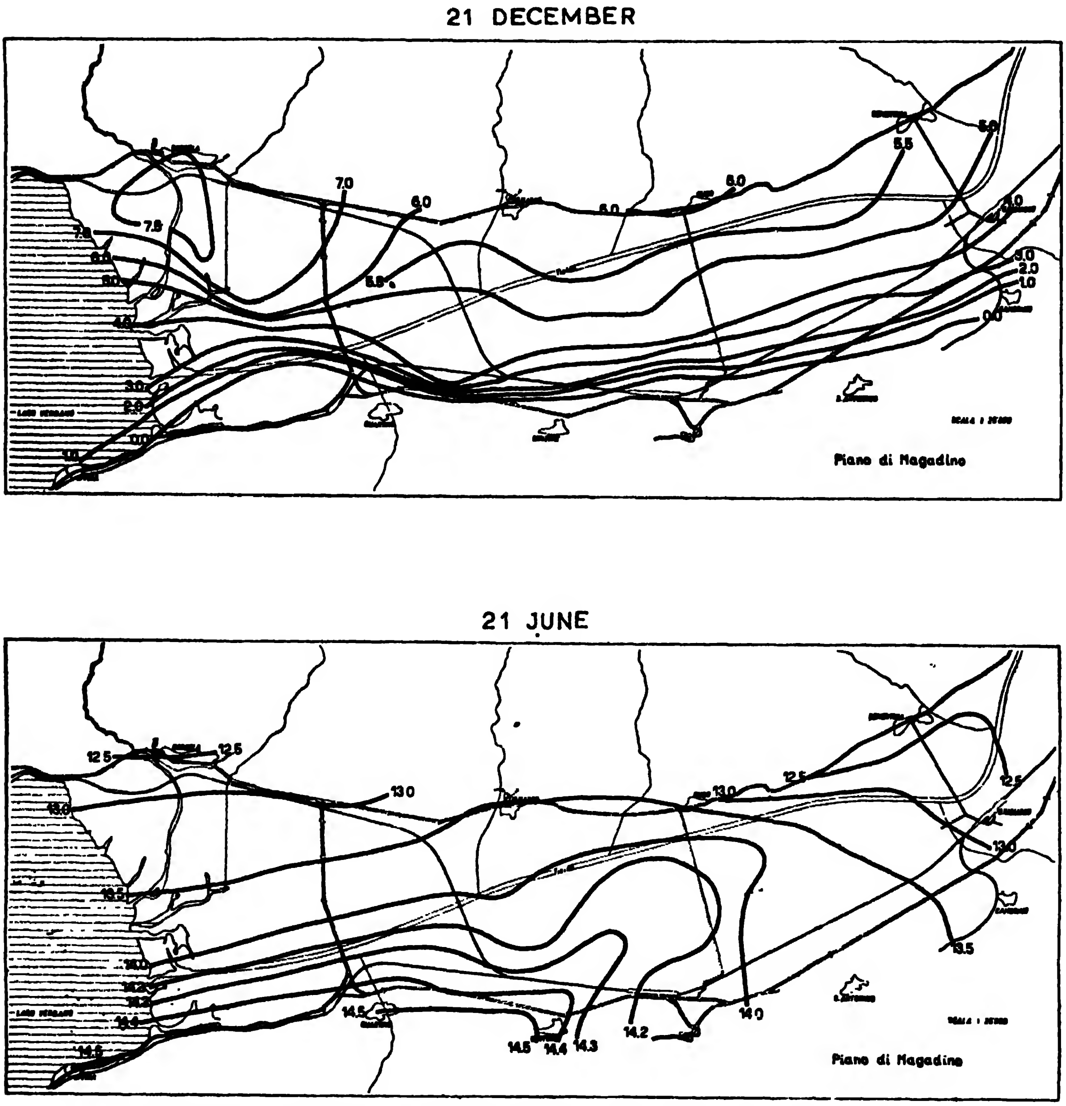

Fig. 2. Isohels of the effective possible duration of sunshine on the plain of Magadino.

Direct solar radiation

The intensity of direct solar radiation is the only value which is not influenced by the topography of the terrain, and the Alps therefore provide an exceptional opportunity for determining the dependence of radiation on height above sea-level, both in various spectral regions and in its entirety. The elevation of the horizon needs only be taken into consideration here if one wishes to calculate daily totals of radia-

tion without regard to the angle of incidence or the orientation of the radiated surface.

Radiation of sun + sky, diffuse radiation and circumglobal radiation

The influence of the horizon line is much more decisive in the case of radiation of sun + sky, of diffuse radiation of the sky alone, and of circumglobal radiation,

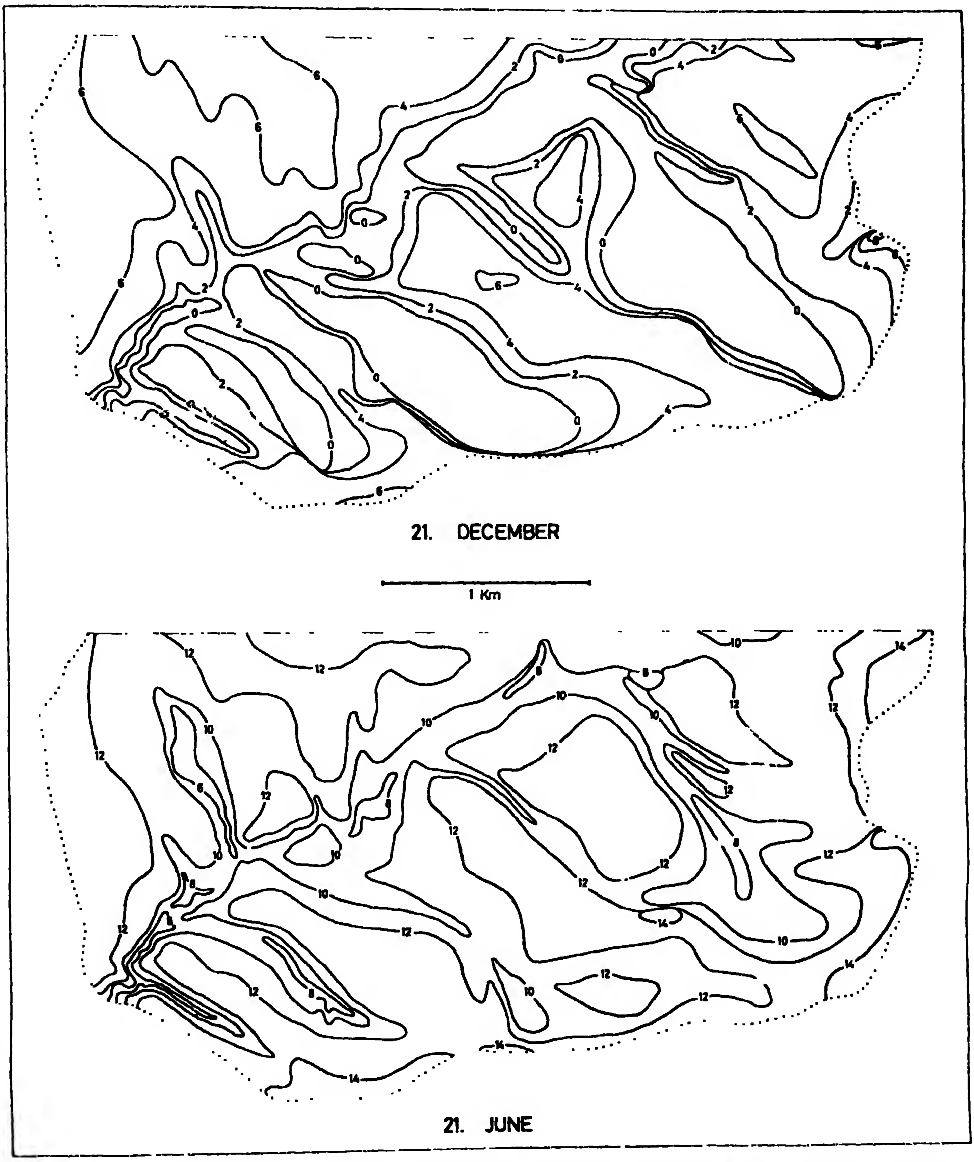

Fig. 3. Isohels of the effective possible duration of sunshine in the region of Baye de Montreux.

because not only do the sun's orbits become shortened through elevations of the horizon, but a particular area of the sky is permanently covered. If the effect of this cover amounts only to a small percentage when the sky is deep blue and of relatively low

References p. 91

intensity, it reaches figures which can by no means be neglected when there is haze or more particularly cloud. It then does not even matter greatly in which quarter the mountain cover lies, since with even cloud radiation is so strongly and so homogeneously scattered that it is not markedly heavier from any particular side (Fig. 5). How closely the screening effect is connected with the proportion of diffuse radiation of the

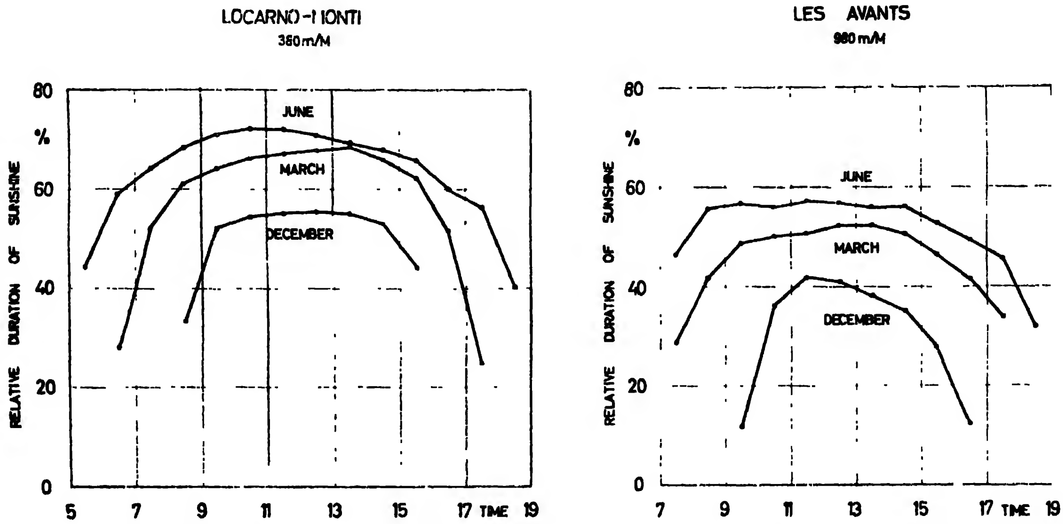

Fig. 4. The diurnal variation of the relative duration of sunshine in the Alps at Locarno-Monti (380 m) and at Les Avants (980 m).

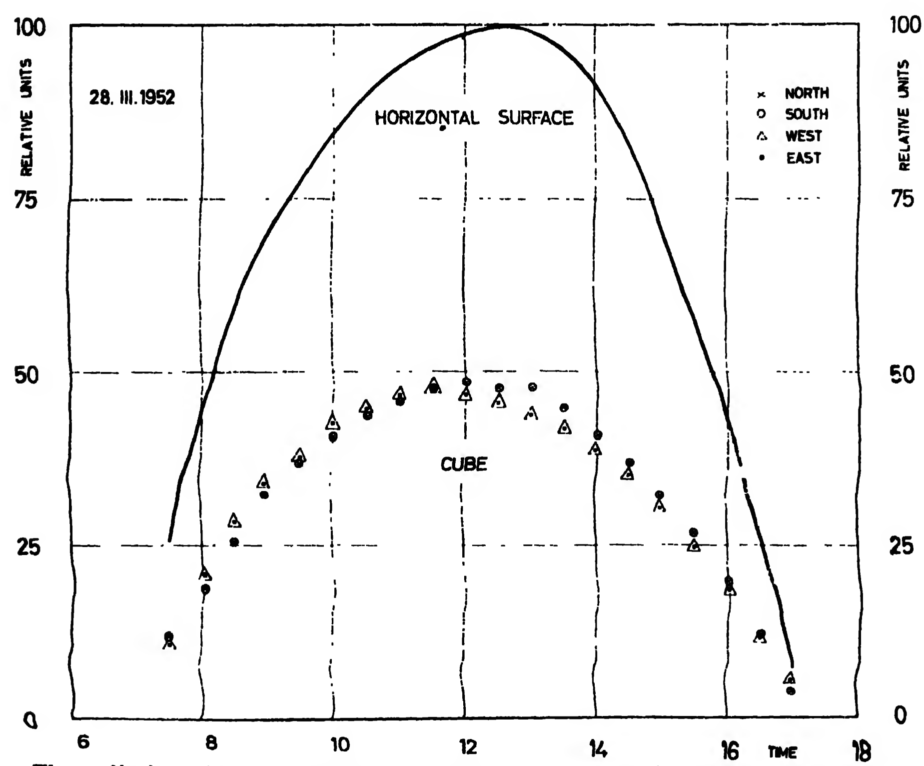

Fig. 5. The radiation of sun + sky on a horizontal surface and on a cube on a day with homogeneous cloudiness (As) at Locarno-Monti.

sky in the total radiation can be shown from an example of circumglobal radiation[3] (Fig. 6). Here a part of the northern horizon was covered which did not effect the length of the sun's orbit. As between a cloudless and a covered sky the radiation loss increased from 3 to 15% on a yearly mean.

The configurations of the horizon are so extremely varied in the Alps that it is extraordinarily difficult to attain strictly comparable values for the elements of radiation wherever diffuse radiation is involved. It is as easy to establish, for instance, the relationship of direct radiation to height as it is difficult to establish this same value for global radiation, diffuse radiation of the sky, and circumglobal radiation. One possible solution would be to work with artificial horizons. In addition to the dominating influence of the topography there is also that due to the direction of slant. Large horizontal surfaces are rare in the Alps, particularly at high altitudes. The impact of radiation differs from that on a horizontal surface, however, according to the degree to which the plane is slanted and orientated. This is equally true, of course, both for solar radiation and for global radiation. As an example the global radiation of a 25° slope facing south may be cited (Fig. 7)[4]. Diffuse radiation plays an important role here to the extent to which it evens out the differences of various slope slants and directions.

Summarizing, we may affirm that the shape of the terrain and the consequent elevation of the horizon, together with the direction in which the terrain slants, act as constant factors very considerably on the radiation climate. From what has been said here it should also be clear that the topography and disposition of terrain is so varied in the Alps that we can only succeed in representing the radiation climate of individual places and not of whole regions. Thus the aim of geographical climatology, which is to

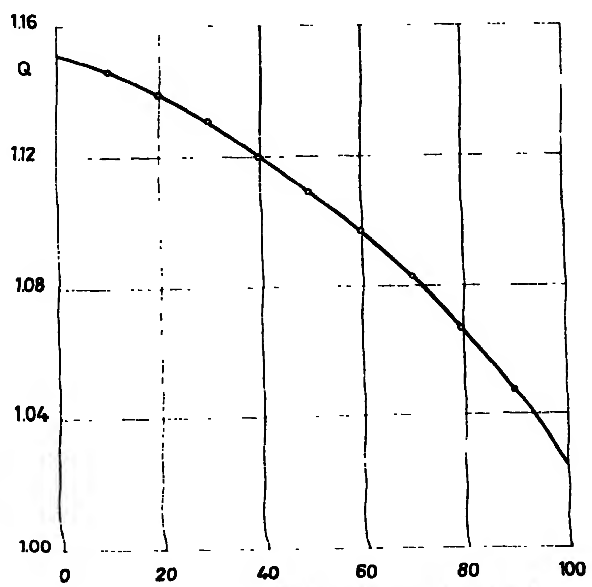

Fig. 6. The screening effect of parts of the sky as a function of the relative duration of sunshine at Locarno-Monti.

References p. 91

represent important elements in cartographical form, here largely proves to be unattainable.

II. THE ALTERATIONS IN WEATHER BROUGHT ABOUT BY THE ALPINE BARRIER

Flohn, Lauscher and Schüepp[5] have attempted to represent the weather conditions for Central Europe on the basis of a kind of dynamic climatology, investigating primarily the effect of the more important general weather situations on the course of the weather in individual areas with their peculiar topography. Hoinkes[6] has also indicated how extraordinarily complicated the relationships are between small-scale and large-scale circulations in the Alps. We cannot hope to show here in detail the way in which the course of the weather is modified by the Alps. We shall deliberately concentrate on a few processes which essentially alter the radiation climate.

The first thing to be mentioned is the marked fluctuating interaction between "föhn" and "stemming" situations, which not only influences the radiation climate but also has a decisive effect on the whole climate both north and south of the Alps, and in conjunction with the situation and shape of the terrain produces distinct climatic oases. With regard to its effect on radiation conditions, the phenomenon of the northern föhn in Switzerland has already been the object of particular study. Series of measurements are available for many years, together with special investigations during the International Geophysical Year.

It is well known that when cold-air masses are brought in by north-west air currents

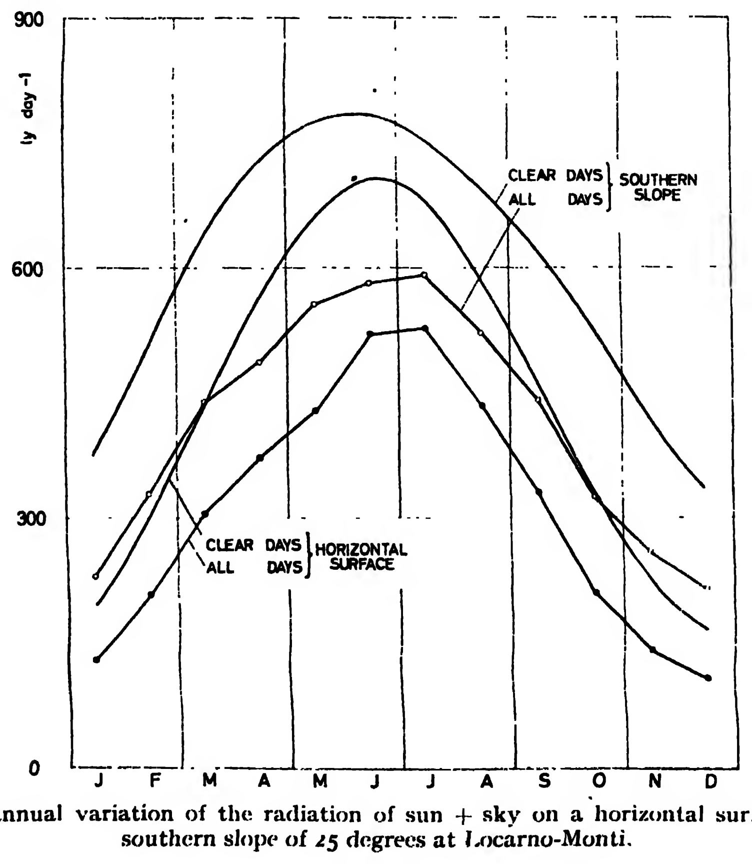

Fig. 7. The annual variation of the radiation of sun + sky on a horizontal surface and on a southern slope of 25 degrees at Locarno-Monti.

the pressure on the northern side of the Alps rises, while on the southern side it drops even in the low-lying areas. Pressure gradients are produced which can be as great as 10 mb/100 km. As the cold-air masses flow over the Alpine ridge they lose the major part of their humidity through being forced upwards, and then under the influence of adiabatic warming they penetrate into the southern Alpine foothills and

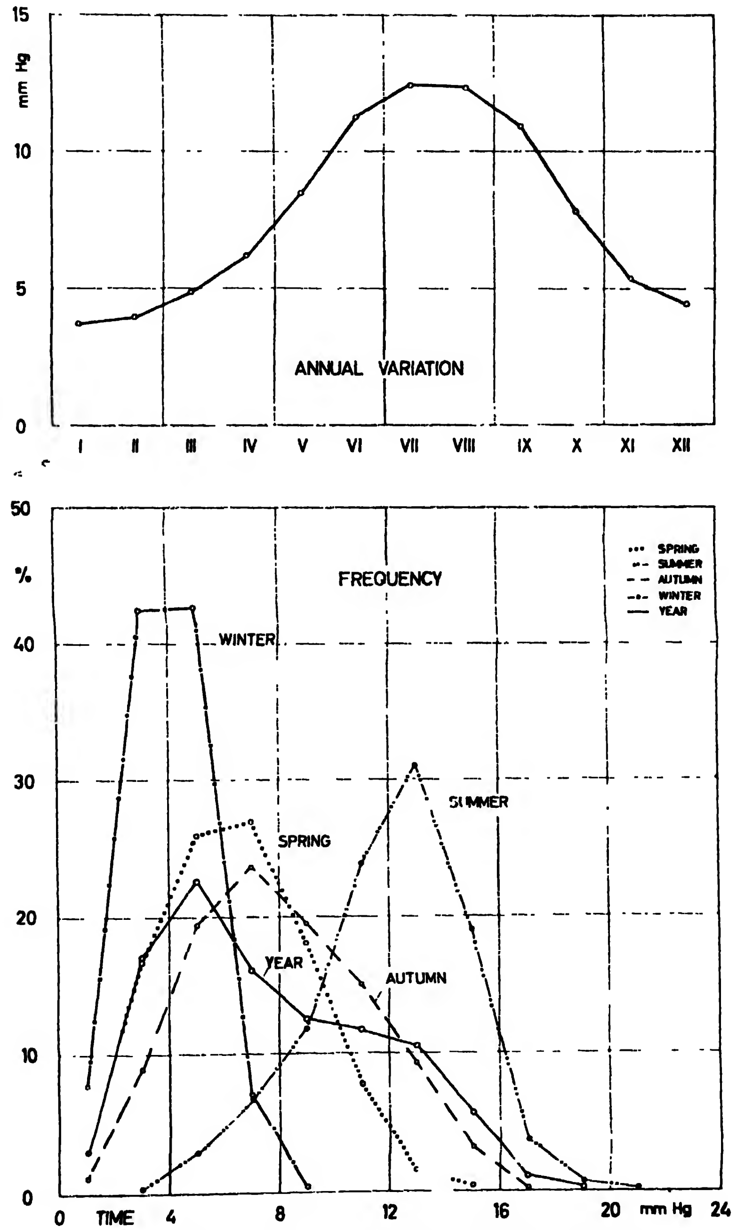

Fig. 8. Vapour pressure at Locarno-Monti (380 m).

on into the Po plain. The air masses may be dried to a very considerable extent, depending naturally on the area in which they originated. Thus, on the southern side of the Alps at a height of 400 m and at an air temperature above 0° vapour pressures

have frequently been recorded of less than 1 mm Hg, while pressures of less than 2 mm Hg are altogether usual (Fig. 8). The air is then extraordinarily pure and transparent, so that visibility seems to be almost unlimited.

The effect of a northern föhn can best be demonstrated by an actual example. During the International Geophysical Year numerous measurements of the intensity of direct solar radiation were made simultaneously at three places on the southern foot of the Alps — at 380, 1040, and 1630 m above sea-level. Let us take a day in June and represent intensity as a function of the time of day (Fig. 9). Apart from the

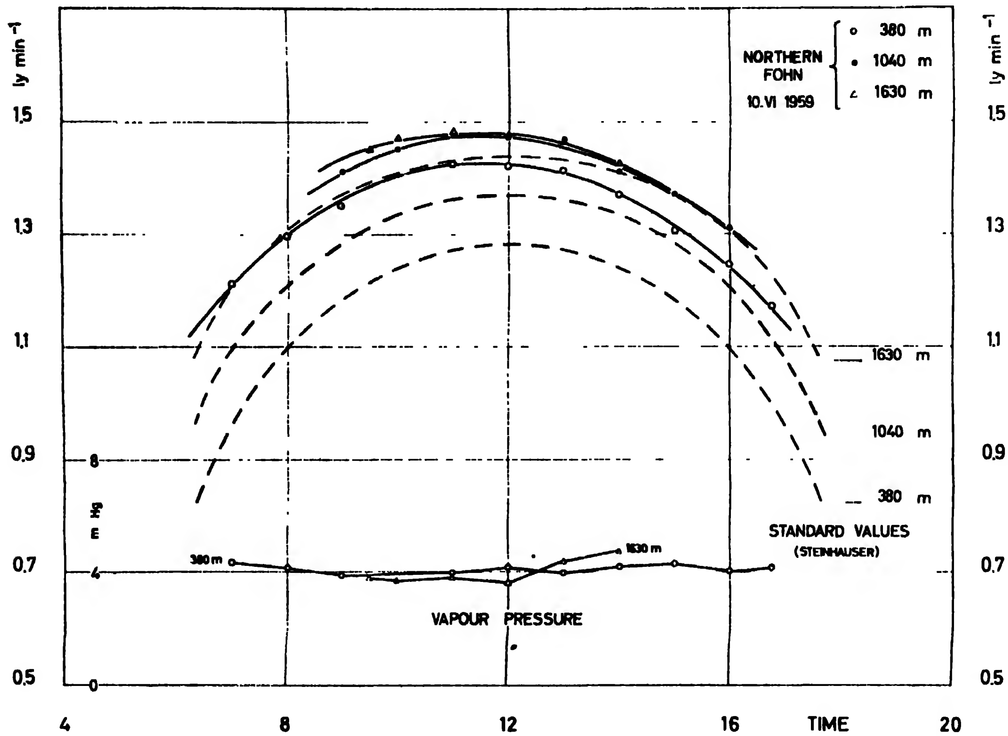

Fig. 9. The influence of the föhn on the intensity of the direct sun radiation (southern slope of the Alps).

absolute high values it is surely striking how closely the curves follow one another for the different heights. If on top of this family of curves is plotted the intensities which according to the investigations of F. Steinhauser are to be expected on average for these heights, then it appears that the curve for 380 m with northern föhn coincides with the curve for 1630 m under normal conditions[7]. The vapour pressure on this day (June 10th, 1959) was approx. 4 mm at both heights, which is an extraordinarily low value for 380 m above sea level at an air temperature of around 20°. The intensity of direct solar radiation at the southern foot of the Alps has been regularly recorded for more than two decades, and from these measurements the following picture emerges: on days when there is northern föhn, and also in certain anticyclonic situations (föhn of the free atmosphere), degrees of intensity are reached at only a few hundred meters which correspond to a height of more than 1600 m[8,9]. In Fig. 10 records over a long

period of direct solar radiation at Davos and Locarno-Monti have been collated which make this situation clear. Since northern föhn is a very frequent phenomen except during the actual summer months, the entire radiation climate is considerably affected by it and something resembling a radiation oasis is created. To what extent

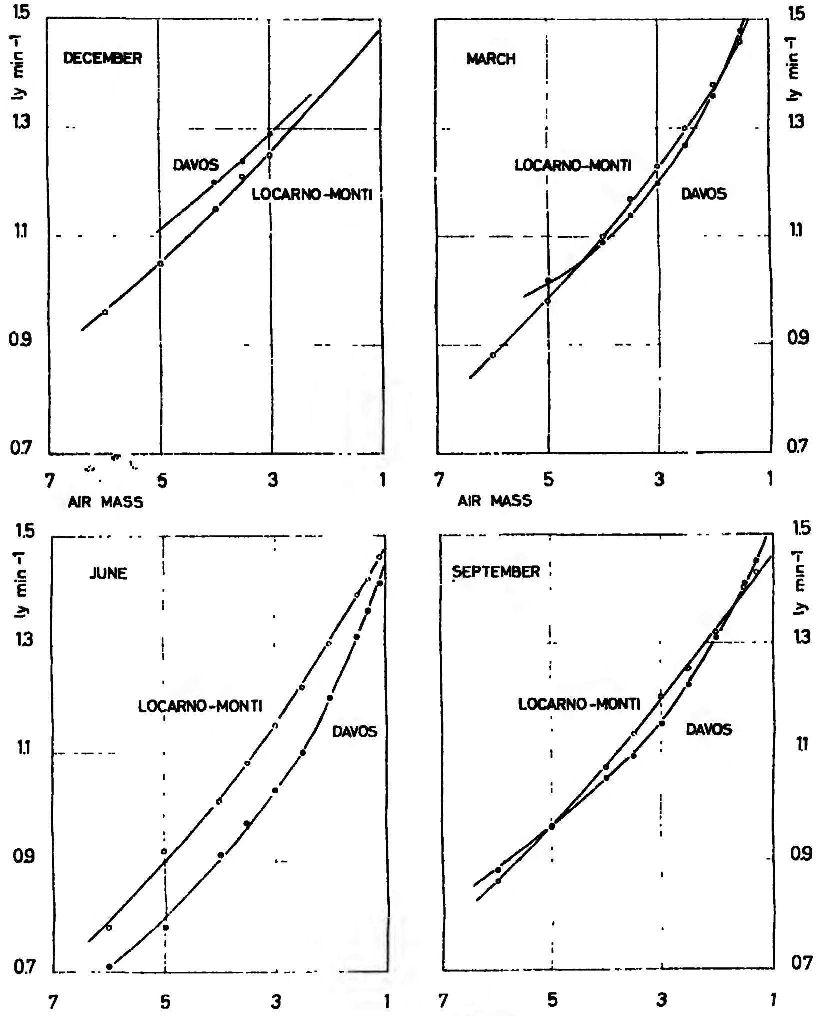

Fig. 10. The intensity of the direct solar radiation on days with northern föhn at Locarno-Monti (380 m) and at Davos (1560 m). Average values.

the same effect operates also for southern föhn, which would affect the northern slopes of the Alps, has still to be examined. Unfortunately, in Switzerland there are not adequate records for this purpose.

For northern föhn numerous experiments have also been made on the intensity of direct solar radiation in various spectral regions (yellow and red filter), but the results are still under analysis.

References p. 91

The purity and lack of water vapour in the air which are brought about by northern föhn naturally have an effect on all other elements of radiation. They increase the components of solar radiation in the case of radiation of sun + sky, whereas they considerably reduce those of diffuse radiation of the sky. The smallest values for diffuse radiation of the sky are found during northern föhn.

What is true for global radiation is true also for circumglobal radiation. As a result of the large proportion of solar radiation in global radiation, or alternatively in circumglobal radiation, the maximum values here too belong to northern föhn situations or anticyclonic situations. It is thus not the case that the decreased components of solar radiation under haze conditions are always entirely compensated by increased diffuse radiation of the sky. Apart from the actual föhn wall along the

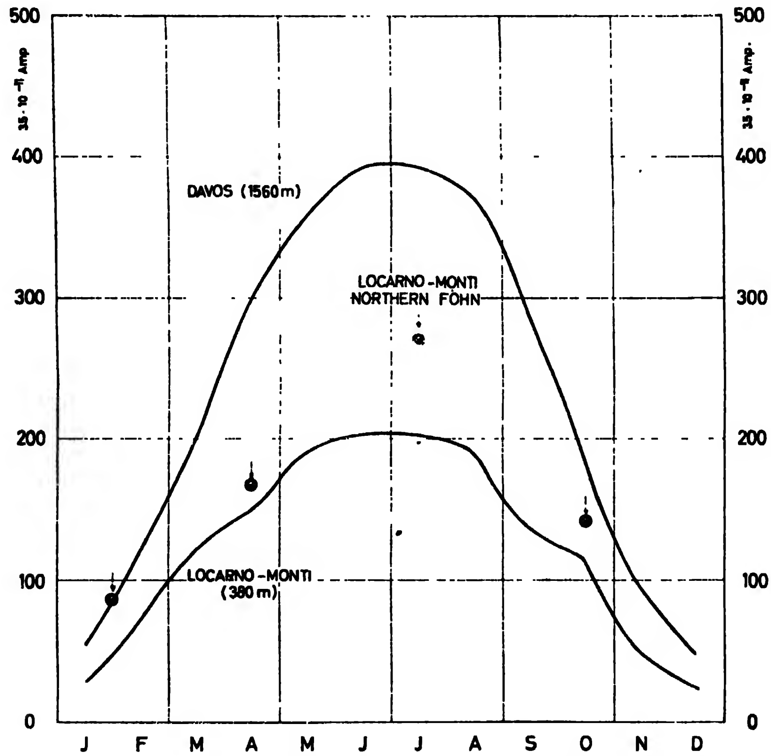

Fig. 11. The influence of the northern föhn on ultraviolet radiation of the sun (midday-values).

Alpine ridge, diffuse radiation of the sky can undergo very considerable increase as a result of cloud in northern föhn situations. Föhn clouds are often brilliantly white and particularly in the neighborhood of the sun they have a remarkable power of radiation. We shall have occasion to return to this problem later.

The maximum degrees of intensity of heat radiation from the sun, which occur in northern föhn situations, are not found in ultraviolet radiation, although here too considerable variations occur according to the degree of purity of the atmosphere. The highest intensity ever measured at the southern foot of the Alps at about 400 m above sea level was 275 Davos units, whereas the average intensity for June in Davos

amounts to nearly 400 D.u. (Fig. 11)[10,11]. The explanation for this difference is to be found in the fact that the intensity of ultraviolet radiation is primarily conditioned by the length of the atmospheric layer penetrated, whilst the total radiation of the sun is dependent above all on the vapour and haze content of the air.

Let us return to the northern föhn situation and examine its effect on cloud conditions. Air masses which are descending and increasing in temperature adiabatically have obviously a strong power to dispel cloud and, without föhn, cloud formations and therefore also the radiation climate would be quite different on both the north and south sides of the Alps. In the peak districts and generally also in the passes föhn and "stemming" situations produce the same effect, however, — an almost

Fig. 12. Lenticular wave clouds during a situation of northern föhn at Locarno-Monti.

closed cloud ceiling. The kind of cloud which forms is also modified by föhn. In descending masses of air mid-level clouds are chiefly found, amongst them the characteristic *Altocumuli lenticularis* (Fig. 12). In the valley areas of the föhn zones closed cloud banks are rare. This is important for the radiation climate inasmuch as diffuse radiation increases sharply up to a certain boundary value of cloud density and beyond it decreases again (Fig. 13). At about 400 m our investigations show this value to be approx. 5/10, at 2000 m Dirmhirn[12] gives about 9/10.

The counterpart to the descending air masses is the "stemming" situation, where compact masses of cloud cause a considerable decline in radiation. Duration of sunshine and direct solar radiation decrease to 0, while only about 12% or less of the global radiation possible on cloudless days is allowed to pass in the low-lying areas[13].

References p. 91

Unfortunately no systematic investigation into the power of "stemming" situations to cause cloud layers in the Alps has been carried out, so that no comparison is possible with conditions in non-mountainous areas.

It would be of great interest to know the entire cloud pattern over the Alps in föhn and "stemming" situations. This could not possibly be achieved, of course, on the basis of observations from individual stations, but modern high-altitude photo-

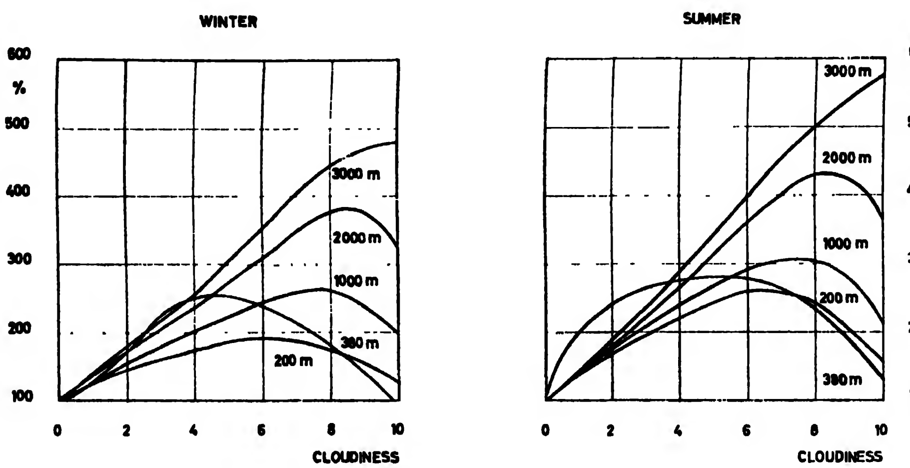

Fig. 13. The relation between sky radiation and cloudiness on different altitudes.

graphic techniques make it a feasible project. Certainly the influence of a mountain range on air currents and therefore on cloud fields extends much higher and further than used to be supposed. Published material gives the height of influence at ten times the relative height, and the breadth of the föhn and "stemming" areas at fifty times the relative height[5].

Although föhn and "stemming" situations are the most impressive examples of weather processes brought about by the Alps, they are of course not the only ones. Every movement of fronts and air masses is modified by the Alps to a greater or lesser extent and influences radiation conditions[14]. The Alps constitute a mighty barrier against cold air masses from the northern sector, a fact which shows up not only in the smaller quantity of cloud but also in the predominance of medium cloud at the southern foot of the Alps. Amongst the large-scale factors should be mentioned primarily thunderstorms, which become intensified on the southern Alpine slopes through the forcing upwards of moist-labile air masses. They chiefly work themselves out in the outer Alpine regions. About 25% of all thunderstorms at the southern foot of the Alps occur in "stemming" situations[15]. But it is also important not to underestimate the role played by local thunderstorms which are closely associated with particular topographical conditions. If one wishes, however, to determine the influence of these storms on the radiation pattern, one must take into account the diurnal variations in such storm behaviour, in the same way as with other meteorological elements. It is clear, in fact, that storm activity and therefore also precipitation are at their maximum during the evening and night[16,17]. Thus, on the southern

Alpine slopes we find the climatological paradox that heavy precipitation can coexist with long duration of sunshine. Heavy rainfall during the night has a purifying effect on the air, because it washes away dust particles, with the result that very high degrees of radiation intensity are often recorded on the morning following thunderstorms and heavy rain.

Amongst the phenomena which determine the radiation climate we should also mention particularly orographic clouds, which are not only important in slope and peak areas but extend far beyond the actual mountain range affected, taking the form then of wave clouds. The slope cumuli which form mainly in the summer also raise noticeably the intensity of diffuse radiation in valley areas.

One further influence should not be forgotten, – namely, that due to the local systems of winds which are extremely numerous in the Alps. The valley and mountain winds which blow up and down the large valleys, together with the slope winds, bring about a thorough mixing of the air masses, and as a result the transmission of radiation through the valley air is increased. If the air stagnates, highly developed layers of

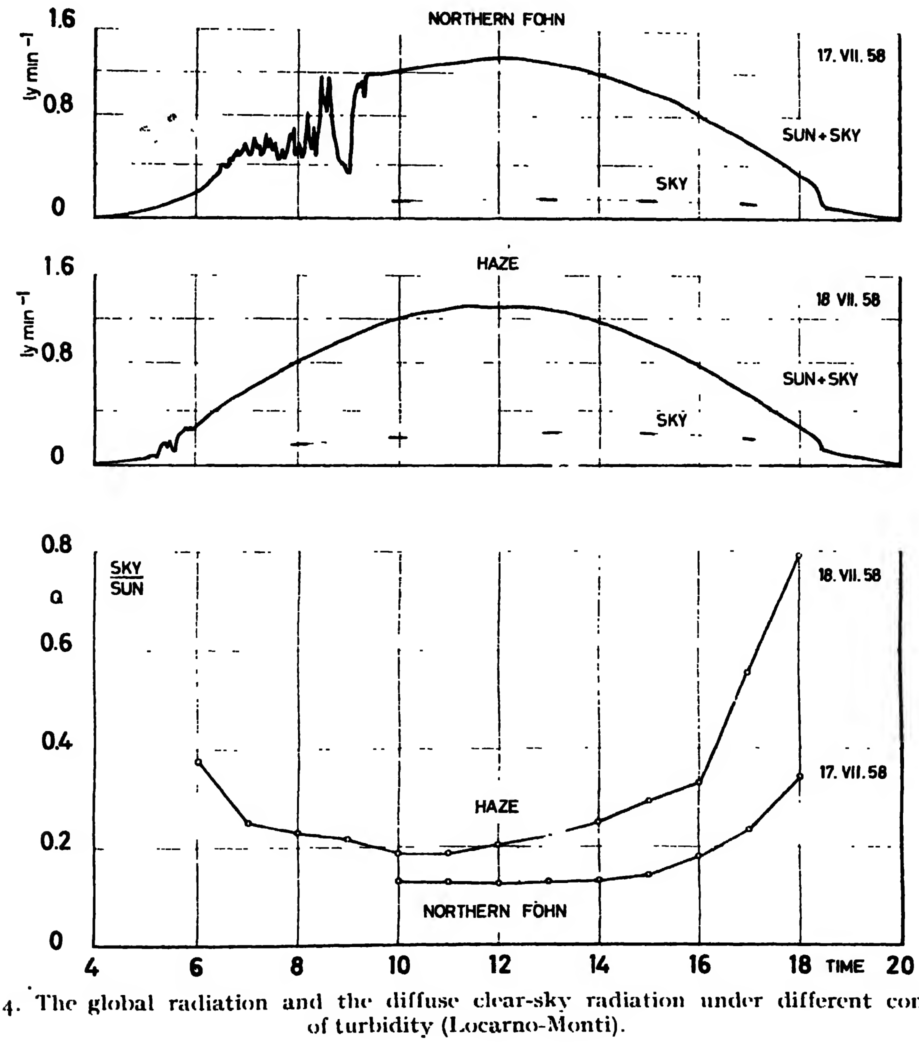

Fig. 14. The global radiation and the diffuse clear-sky radiation under different conditions of turbidity (Locarno-Monti).

References p. 91

haze and dust can form in the valleys, which may last for weeks according to the weather situation, particularly in the winter months when there is only slight vertical movement of the air masses. But even in the summer months stagnating air masses are found in valleys that are very largely enclosed. They naturally affect above all the intensity of direct solar radiation, and also that of global radiation. In the case of global radiation the total intensity of sun and sky on days of heavy haze is often the same as the total for clear days, but the proportions of solar radiation and radiation from the sky are very different. A characteristic example of this on the 17th and 18th July 1958 at the southern foot of the Alps is given in Fig. 14. If one draws the relationship of radiation from the sky to solar radiation for both days, it becomes even clearer how greatly diffuse radiation is increased by haze. In passing it may also be observed that smoke-producing industries which are located in valleys can very much alter the radiation climate.

We have already discussed the influence of the aspect or "slant" of terrain. This effect can, however, be considerably altered by the condition of the ground surface, — in particular when it is covered with snow. Already at 1600 m the proportion of snow in the overall amount of precipitation is 50%. As is well known, the reflecting power of snow varies between wide limits. Old snow has an albedo of about 60%, but this value can rise to 90% in the case of fresh snow. Reflection from snow operates in two ways:

(1) through radiation from the mountain-side and the valley floor,

(2) through multiple reflection, *i.e.* mutual reflections between snow and clouds, of the kind indicated particularly by Götz[18]. The additional radiation from the mountain slopes has a perceptible effect on global radiation; the total reflection of the snow can be much better comprehended from a biological point of view with regard to circum-global radiation, which we can nowadays measure in such an elegant manner by means of the Bellani distillation lucimeter[19,20].

Multiple reflection is particularly striking when there is homogeneous cloud, when it can reach remarkably high values. Very large interdiurnal variations occur when a relatively thin layer of snow melts away within a few hours, due perhaps to föhn, and the only reflection then is from grass, forest, and stone surfaces. The whole problem of albedo in its bearing on the balance of radiation is most complex, and far too little understood to allow any quantitative statements to be made[21]

If we review all the factors which influence the radiation climate of the Alps, then we see that it is not simply the absolute values of the various radiation elements which must be taken into account, but above all the degree to which these values vary. Whether we stand at the northern foot of the Alps, or at the southern foot, or in the central massif itself, we find a distinctly bracing climate with regard to radiation.

We have here been able only to sketch in rough outline the effect of the Alps on the radiation climate. In order to gain a complete picture, two conditions would have to be fulfilled:

(1) extension of the network of stations at which radiation is measured,

(2) the most thorough understanding possible of the individual elements of radiation.

Above all, close cooperation with the new study of synoptic meteorology should be aimed at, a branch of research which is unfortunately still in its infancy.

REFERENCES

1 J. C. THAMS AND E. ZENONE, *Landwirtsch. Jahrb. Schweiz*, 66 (Neue Folge 1) (1952) 139.
2 J. C. THAMS, *Arch. Meteorol. Geophys. u. Bioklimatol.*, Ser. B, 6 (1955) 417.
3 J. C. THAMS, *Geofis. pura e appl.* 45 (1960) 303.
4 J. C. THAMS, *Arch. Meteorol. Geophys. u. Bioklimatol.* Ser. B, 7 (1956) 190.
5 H. FLOHN, F. LAUSCHER AND M. SCHÜEPP, *Witterung und Klima in Mitteleuropa*, 2nd ed., Zürich, 1954.
6 H. HOINKES, *Arch. Meteorol. Geophys. u. Bioklimatol.* Ser. A, 5 (1953) 304.
7 F. STEINHAUSER, *Meteorol. Z.* 56 (1939) 172.
8 J. C. THAMS, *Arch. Meteorol. Geophys. u. Bioklimatol.* Ser. B, 6 (1954) 139.
9 W. MÖRIKOFER, *Festschr. 110. Jahresversammlung S.N.G.*, *Davos*, (1929) 33.
10 FL. AMBROSETTI AND E. ZENONE, *Geofis. pura e appl.*, 15 (1–2) (1949)
11 F. LINDHOLM, *Festschr. 110. Jahresversammlung S.N.G.*, *Davos*, (1929) 5.
12 I. DIRMHIRN, *Arch. Meteorol. Geophys. u. Bioklimatol.*, Ser. B, 2 (1951) 301.
13 J. C. THAMS, *Geofis. pura e appl.* 14 (1–2) (1949).
14 M. BOUËT, *Catalogue des fronts en Suisse pendant l'année polaire internationale* 1932/1933. *Année polaire internationale* 1932–1933, *participation suisse*, Publication de la Station centrale suisse de Météorologie à Zürich, 1941.
15 *Schlussbericht der Eidg. Kommission zum Studium der Hagelbildung und der Hagelabwehr über den Grossversuch Nr. I zur Bekämpfung des Hagels auf der Magadinoebene (Tessin)* 1948–1952, Zürich 1955.
16 J. C. THAMS, *Wasser- u. Energiewirtschaft*, 3 (4/5) 1948.
17 FL. AMBROSETTI, M. BIDER AND M. BOUËT, *Arch. Meteorol. Geophys. u. Bioklimatol.*, Ser. B, 8 (1957) 171.
18 F. W. P. GÖTZ, *Klima und Wetter in Arosa*, Frauenfeld, 1954.
19 P. COURVOISIER AND H. WIERZEJEWSKI, *Arch. Meteorol. Geophys. u. Bioklimatol.*, Ser. B, 5 (1954) 413.
20 J. C. THAMS AND H. WIERZEJEWSKI, *Arch. Meteorol. Geophys. u. Bioklimat.*, Ser. B, 9 (1958) 185.
21 F. SAUBERER AND O. HÄRTEL, *Pflanze und Strahlung*, Leipzig, 1959.

COMPARISON OF SOLAR RADIATION IN THE NORTHERN AND SOUTHERN SUBTROPICS WITH CONDITIONS IN THE TEMPERATE CLIMATES

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SUMMARY OF LECTURE

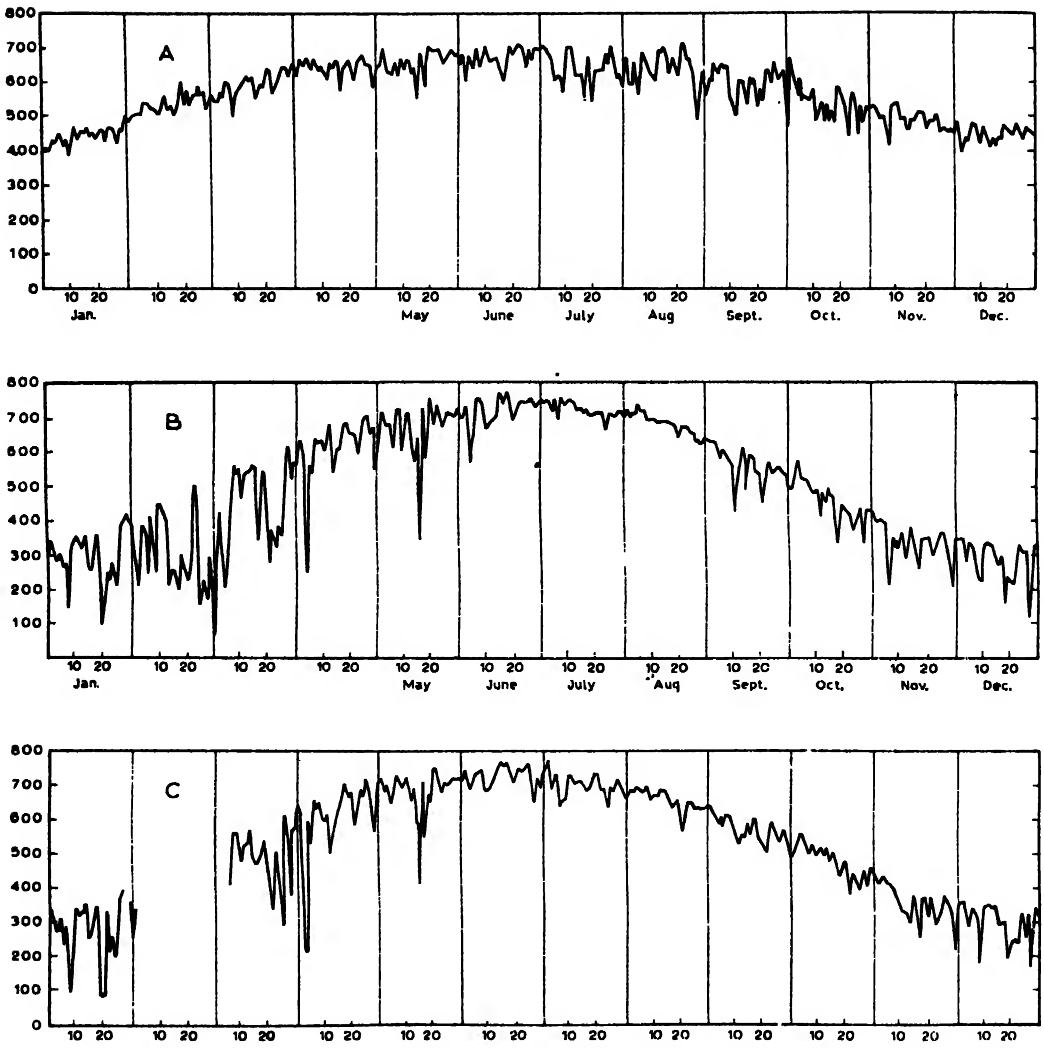

Fig. 1. Daily global solar radiation (cal/cm²·day). A, Wake Island (Pacific Ocean) $\varphi = 19°48'$ N. $\lambda = 166°35'$ E.; B, Jerusalem, 1959; C, Lod.

Radiation measurements are available from stations located in Arizona, New Mexico and California, the subtropical parts of the United States. Their agreement with data obtained from Jerusalem is very good. In both of these regions, solar radiation attains

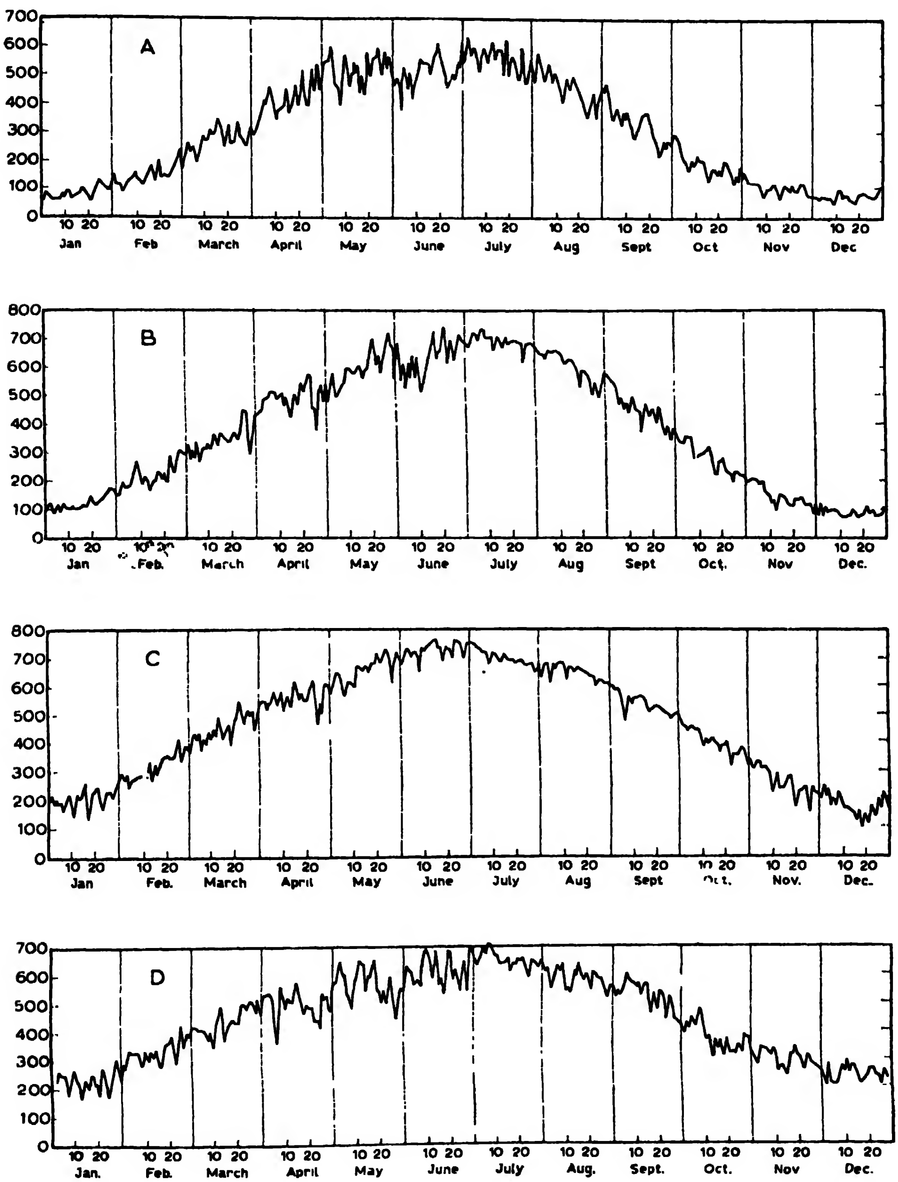

Fig. 2. Daily global solar radiation (cal/cm²·day). A, Seattle; B, Medford, Oreg.; C, Fresno; D, Los Angeles.

values of 750 cal/cm² · day on long summer days (14 h), and in winter drops to about 270 cal/cm² · day.

The subtropical summer in Jerusalem is clear, cloudless and dry, and the monthly radiation is as high as 22,000 cal/cm², while in winter, which not only has shorter days, but also is the cloudy and rainy season, radiation reaches values of 8500 -9000 cal/cm²·month.

References p. 101

TABLE I

JERUSALEM HOURLY GLOBAL SOLAR RADIATION (SUN & SKY ON HORIZONTAL SURFACE) (cal/h·cm²)

month	5/6	h 6/7	h 7/8	h 8/9	h 9/10	h 10/11	h 11/12	h 12/13	h 13/14	h 14/15	h 15/16	h 16/17	h 17/18	h 18/19	daily total
1953															
J		0.5	4.0	16.4	31.6	44.3	55.4	63.4	52.5	39.4	23.0	4.5	0.5		334.1
F		2.0	11.3	29.8	45.0	49.6	52.8	56.0	51.5	44.2	30.8	17.0	1.5		394.5
M	1.0	8.0	20.0	40.4	62.2	64.0	74.4	71.0	57.0	52.5	47.0	32.0	12.0	0.5	542.4
A	2.5	12.5	31.4	50.0	68.0	77.0	83.0	82.0	76.3	63.7	45.2	33.0	12.4	2.2	639.2
M	3.9	19.6	32.5	50.9	69.3	79.3	80.6	88.4	72.0	67.0	53.8	37.1	18.9	5.8	671.1
JE	7.1	22.1	40.1	58.7	74.5	87.9	91.1	91.7	86.4	75.3	60.5	43.2	23.6	7.0	769.2
JY	4.7	22.5	42.1	59.5	73.8	84.0	88.9	87.7	81.7	72.8	58.1	40.4	20.7	4.5	743.6
A	2.3	17.4	35.5	54.3	68.8	78.2	82.0	83.5	76.4	66.5	51.8	34.7	15.1	1.1	668.2
S	0.7	8.8	29.3	48.2	64.4	71.4	79.3	79.3	71.3	60.6	44.7	24.8	7.0	0.6	593.4
O		3.0	16.3	32.8	50.0	60.4	62.1	62.4	57.7	46.3	32.0	14.0	2.0		439.4
N		1.6	10.3	24.0	38.2	47.0	49.3	47.4	40.9	33.0	22.0	9.4	0.9		324.0
D			4.4	14.2	23.9	33.4	41.0	40.1	36.0	25.1	14.2	4.8			237.1
1954															
J		0.5	5.6	19.4	30.7	45.2	49.7	50.3	45.7	30.5	21.3	5.3	0.5		305.0
F		3.0	8.7	27.0	38.2	47.0	47.5	46.4	42.8	36.4	22.2	9.0	1.0		329.4
M		4.0	19.3	38.1	51.1	62.0	61.9	61.6	56.7	47.1	36.3	17.7	4.2		460.0
A	1.9	11.0	29.4	43.9	58.1	64.4	67.5	65.4	62.7	55.7	42.3	26.7	10.4	1.2	540.0
M	2.6	17.1	34.3	57.8	67.3	79.2	83.9	82.4	77.8	68.4	53.5	34.0	16.1	2.2	676.6
JE	6.0	23.4	43.3	60.5	73.4	83.4	88.4	87.8	82.2	71.9	58.7	40.7	21.4	4.3	745.4
JY	4.6	21.9	41.6	59.2	72.2	82.0	87.0	85.9	80.0	71.0	58.0	40.0	20.3	3.9	728.0
A	1.8	15.6	34.0	53.3	67.8	78.5	84.7	83.5	78.6	66.0	53.3	33.7	15.3	1.3	668.0
S	0.9	9.5	29.3	47.9	62.2	73.7	79.5	77.1	70.9	59.4	45.4	26.4	8.1	0.4	591.0
O		2.7	17.6	35.2	51.3	62.3	67.9	66.7	57.9	45.7	33.5	16.7	3.2		460.7
N		0.5	8.7	23.4	37.7	46.1	49.0	50.9	44.8	28.3	18.6	7.8	0.8		317.6
D			4.9	16.0	28.0	34.8	40.1	39.5	35.7	25.4	15.0	5.7	0.5		245.6
1955															
J		0.3	7.5	22.8	38.5	50.0	56.0	55.2	49.5	40.8	21.9	8.5	0.3		351.5
F		1.1	12.1	27.6	45.2	55.4	62.0	60.5	56.3	43.5	29.3	13.7	2.0		409.6
M		5.5	22.9	39.5	52.5	62.6	68.3	64.3	60.2	52.8	37.0	19.8	5.9		491.6
A	1.8	9.2	30.5	45.5	59.2	72.2	76.4	76.4	71.2	62.6	46.7	28.0	11.2	0.7	591.6
M	4.3	17.0	35.4	54.6	70.8	78.5	83.8	83.3	77.9	69.1	57.0	38.6	19.6	3.7	693.6
JE	4.5	23.5	43.2	59.5	72.4	83.0	87.4	88.0	81.7	73.2	60.1	42.0	22.5	5.0	745.6
JY	5.3	24.6	43.0	59.9	72.3	82.2	86.9	86.2	80.4	70.9	58.1	39.7	20.1	3.9	733.5
A	2.1	18.1	37.2	57.0	71.5	81.5	86.2	85.5	80.0	70.2	55.5	36.2	16.6	2.4	700.7
S	0.2	9.2	28.6	47.8	62.5	73.8	78.0	78.0	72.0	61.0	46.6	27.3	8.9	0.3	593.6
O		1.9	19.4	36.9	52.7	61.9	67.6	67.2	60.0	48.4	34.1	16.2	2.9		469.3
N			8.4	22.2	35.9	40.7	44.8	45.0	39.4	31.1	20.5	8.8	1.3		298.1
D			6.2	16.8	28.0	36.6	40.5	41.8	36.6	27.6	17.7	6.7	1.0		259.5
1956															
J		0.5	8.0	21.5	35.8	44.3	49.0	47.1	42.8	34.1	19.2	7.9	1.1		310.4
F		1.7	11.8	27.6	39.1	51.4	55.5	58.8	50.6	37.8	25.0	10.1	1.4		370.8
M		4.3	19.5	31.0	46.0	52.8	59.7	59.1	57.4	46.2	33.9	19.2	6.0	0.6	435.7
A	1.6	9.5	29.1	45.6	62.0	71.2	75.9	75.9	68.3	61.7	47.9	29.6	11.9	1.6	591.8
M	2.0	18.9	41.1	53.7	69.7	79.5	83.5	84.5	80.0	72.2	57.0	38.9	19.0	4.0	704.0
JE	5.3	23.8	42.3	60.5	73.3	82.0	88.7	86.8	82.1	72.9	60.3	43.1	22.4	5.5	742.0
JY	4.0	22.6	40.5	58.9	71.8	80.8	86.7	85.9	80.8	72.9	59.2	40.8	20.9	4.8	730.6
A	0.9	17.1	36.2	55.0	69.6	78.3	85.0	84.3	78.3	68.8	54.3	34.9	15.3	2.0	680.0
S	0.2	10.2	29.8	49.3	65.2	76.2	81.0	81.5	72.4	61.0	46.8	26.4	7.8	0.2	608.0
O		2.5	19.3	37.7	53.7	64.6	67.9	68.3	61.6	50.3	34.5	16.5	3.0		479.9
N		0.4	10.0	25.4	40.0	50.5	54.4	53.5	48.2	38.2	23.6	7.8	0.7		352.7
D			5.2	15.2	26.5	35.3	40.8	39.3	36.7	28.7	16.4	5.9			250.0

TABLE I (continued)

month	h 5/6	h 6/7	h 7/8	h 8/9	h 9/10	h 10/11	h 11/12	h 12/13	h 13/14	h 14/15	h 15/16	h 16/17	h 17/18	h 18/19	daily total
1957															
J			7.8	20.3	31.2	43.2	45.4	43.6	39.3	31.3	20.0	7.2	0.5		292.8
F		0.1	14.1	28.6	42.2	55.9	60.3	58.5	51.0	41.6	30.0	14.4	1.9		398.9
M	0.3	5.3	22.0	34.8	48.8	55.9	55.9	57.8	51.0	44.1	31.0	17.8	6.4	0.1	430.9
A	1.4	13.5	28.0	44.7	58.7	66.8	71.9	71.5	63.9	52.9	41.4	25.6	11.0	1.3	552.6
M	3.8	20.5	38.6	54.0	63.7	72.5	78.5	77.0	72.0	63.7	52.8	35.9	18.6	3.1	654.7
JE	5.0	24.0	42.9	60.2	73.1	82.0	86.6	86.7	81.7	73.4	60.8	43.5	23.6	5.8	752.0
JY	3.8	22.7	40.0	57.3	71.5	80.5	85.2	84.0	80.0	70.7	57.8	42.0	22.6	5.1	782.0
A	2.4	18.2	37.2	55.9	69.0	78.5	83.7	82.8	79.3	67.9	53.4	34.2	15.8	1.8	686.0
S	0.2	8.3	25.9	45.5	60.2	70.8	77.0	75.4	69.8	60.5	45.4	26.9	9.4	0.4	576.0
O		1.9	16.7	30.1	47.4	57.8	62.4	61.9	53.5	48.0	30.2	14.4	2.9		421.2
N		0.2	7.8	19.4	32.8	45.6	52.9	49.1	42.1	31.3	21.2	7.9	1.0		311.4
D			4.5	16.9	30.1	43.7	46.3	44.5	41.8	29.8	16.8	5.0	0.6		281.0
1958															
J			6.0	17.3	29.2	38.4	41.0	41.0	33.8	25.6	17.8	6.2	0.6		256.9
F		1.1	14.2	30.2	45.5	56.1	58.6	50.6	51.0	39.3	29.2	14.0	2.5		401.0
M	0.3	2.3	25.2	40.0	55.6	67.5	72.0	70.9	66.7	56.3	39.0	21.2	5.2	0.5	522.4
A	1.7	6.5	30.8	47.5	61.5	70.8	77.6	76.7	72.7	63.8	47.5	29.6	12.0	1.0	600.1
M	1.9	15.6	42.4	55.6	69.8	79.2	82.0	83.6	79.1	70.0	57.6	39.2	19.6	4.0	700.0
JE	2.5	21.3	43.5	57.7	70.1	80.0	85.8	86.5	80.2	72.7	60.2	43.6	23.7	6.8	735.0
JY	2.0	21.8	42.5	57.1	70.6	79.8	84.1	84.9	80.3	72.5	57.5	42.2	22.5	6.3	725.0
A	1.0	14.1	37.8	54.0	68.0	78.0	82.8	82.2	78.0	70.0	54.5	36.5	17.1		675.0
S	0.3	7.2	29.8	44.6	58.2	71.2	76.0	75.2	69.9	61.7	43.5	25.5	9.3	0.6	572.7
O		1.4	20.2	35.4	51.3	63.4	67.5	68.0	60.1	48.3	34.3	17.2	3.5		408.2
N		0.2	9.2	22.3	38.1	48.8	53.0	50.2	44.0	35.5	22.6	8.1	1.1		332.9
D			5.3	18.0	30.8	38.6	43.0	44.7	40.0	29.9	18.5	5.6	6.7		274.5
1959															
J		0.1	6.8	19.4	35.3	44.2	48.2	46.0	39.4	30.5	18.5	6.1	0.9		295.3
F		1.1	10.7	24.4	37.5	40.0	41.6	46.7	41.2	30.4	21.4	7.7	0.8		299.6
M	0.6	4.7	20.5	39.5	48.6	54.2	58.3	54.7	53.0	45.4	32.4	16.4	4.7	0.4	433.5
A	1.3	10.8	28.9	46.7	62.8	71.8	71.7	76.3	72.2	61.8	49.3	29.0	12.2	1.3	599.1
M	3.2	19.3	36.2	52.9	65.5	77.5	81.9	80.5	73.7	66.3	52.9	36.4	16.6	2.9	665.8
JE	5.5	21.8	39.0	56.8	70.5	80.1	84.7	86.6	78.9	70.3	57.9	38.9	21.3	3.6	716.6
JY	4.6	21.1	39.4	56.7	69.6	78.7	86.0	85.7	79.7	71.9	57.5	40.1	21.5	4.5	717.3
A	2.0	17.6	35.3	53.8	68.1	78.0	82.2	82.6	77.6	68.3	53.4	35.6	16.0	1.9	672.8
S	0.2	10.1	25.6	44.1	57.1	69.0	76.1	73.3	68.7	59.3	44.0	27.2	8.1	0.3	563.1
O		3.3	17.7	33.8	50.8	60.2	63.6	65.0	58.6	46.8	34.0	17.1	3.0		453.8
N		0.2	9.4	24.4	38.1	47.5	51.1	54.8	46.7	35.9	22.3	7.6	0.3		338.3
D			5.5	19.3	33.2	42.1	48.3	47.1	41.2	30.2	17.7	5.0	0.2		289.8
1960															
J			5.6	17.1	31.9	41.3	43.3	43.7	39.5	29.6	18.1	5.5	0.4		276.0
F		0.9	10.2	27.0	42.5	53.5	60.0	56.8	50.5	44.4	28.9	10.6	0.7		386.0
M		3.6	20.1	33.7	51.8	61.2	64.5	65.0	59.9	47.7	32.2	17.0	3.8		460.5
A	1.2	13.7	29.7	47.8	62.9	71.5	77.2	78.8	71.1	59.4	43.3	31.7	9.0	1.3	598.0

Therefore, the annual solar radiation per cm^2 attains a value of 190×10^3–200×10^3 cal of total global radiation, including the diffuse component. The value of the latter is low in summer, being less than 100 cal/cm^2·day, while during the cloudy months it can reach a monthly mean of 200 cal/cm^2·day (max. in April), see Fig. 7.

A comparison of subtropical values between the northern and southern hemispheres shows the results of observations taken in South Africa between latitudes 22°S and 33°S. It is clear that in South Africa there exists some factor which attenuates the

References p. 101

radiation even in high places such as Windhoek and Pretoria. I tend to the opinion that over the subtropical parts of the African continent, in both hemispheres, there is a heavy pall of dust which attenuates radiation. In North Africa this appears obvious, at Tamanrasset in the Sahara, on the Tropic of Cancer, 1400 m above M.S.L.

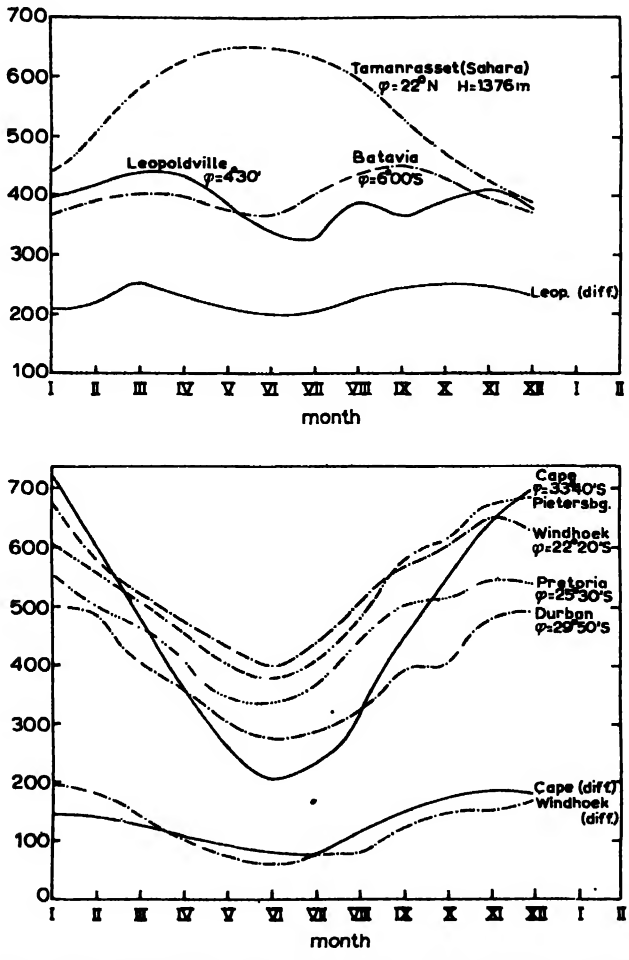

Fig. 3. Daily global and diffuse solar radiation (cal/cm²·day), in South Africa.

as in the South, at Windhoek on the Tropic of Capricorn, at about the same altitude. While discussing Africa, it is of interest to note the radiation near the equator in Leopoldville, Congo, as compared with that of Djakarta, located in the same latitude (6°S). The scattered radiation is much higher here than in either of the subtropics, while the yearly global radiation is about 30% lower than in the subtropics.

COMPARISON OF RADIATION VALUES BETWEEN JERUSALEM AND THE TEMPERATE ZONES

The numerous observations in Europe and North America enable us to draw a clear picture of the radiation distribution compared with that of the subtropics. In summer, because of cloudiness, radiation is weak in the temperate zones in spite of the longer days. The radiation deficiency is even more pronounced in winter as a result of low sun, shortness of the day and the heavy cloudiness. In winter, radiation drops off to

zero as the Arctic Circle is approached. North of the Arctic Circle there is no radiation at all during the winter. On the other hand, recent observations made beyond the

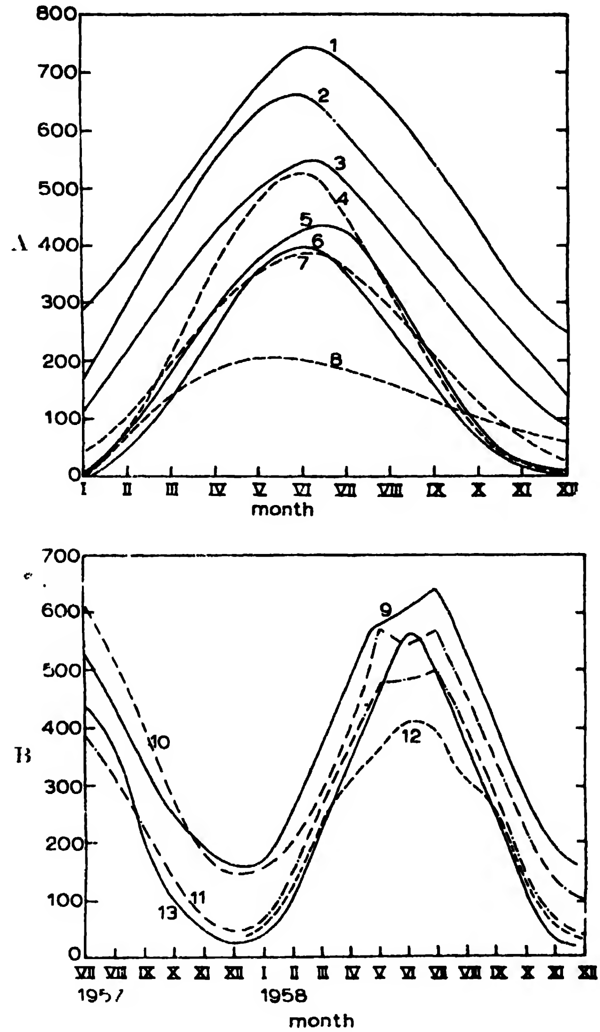

Fig. 4A. Global solar radiation (cal/cm²·day); 1, Jerusalem; 2, Weissflujoch; 3, Davos; 4, Fairbanks; 5, Helsinki; 6, Lerwick; 7, De Bilt; 8, Diffuse.
Fig. 4B. 9, Athens; 10, Rome; 11, Gdynsk; 12, Uccle; 13, Visby.

Arctic and the Antarctic Circles show that in summer there exists definitely high radiation. Liljequist's observations in the Antarctic, and measurements from Alaska on the edge of the Arctic Sea show values for clear 24-h periods which approach those of subtropical radiation.

Recent observations at Kew Observatory and at Lerwick in the British Isles show that there is little variation in radiation between latitudes 50°N and 60°N. The radiation in the south is weakened by industrial pollution of the air, which causes sufficient extinction to reduce radiation to values measured 10 degrees farther north. This effect is also noticeable at De Bilt in The Netherlands and Brussels. On the other hand, observations made at Helsinki and Stockholm (in Europe), and at Fairbanks,

References p. 101

Alaska, show radiation values higher than those of Western Europe. The values measured at Stockholm, Helsinki and Fairbanks are practically identical. On the average, for all types of weather, the summer noon radiation is 60 $cal/cm^2 \cdot h$, and on clear days radiation is even higher. The Russian observations in Europe and Siberia during the

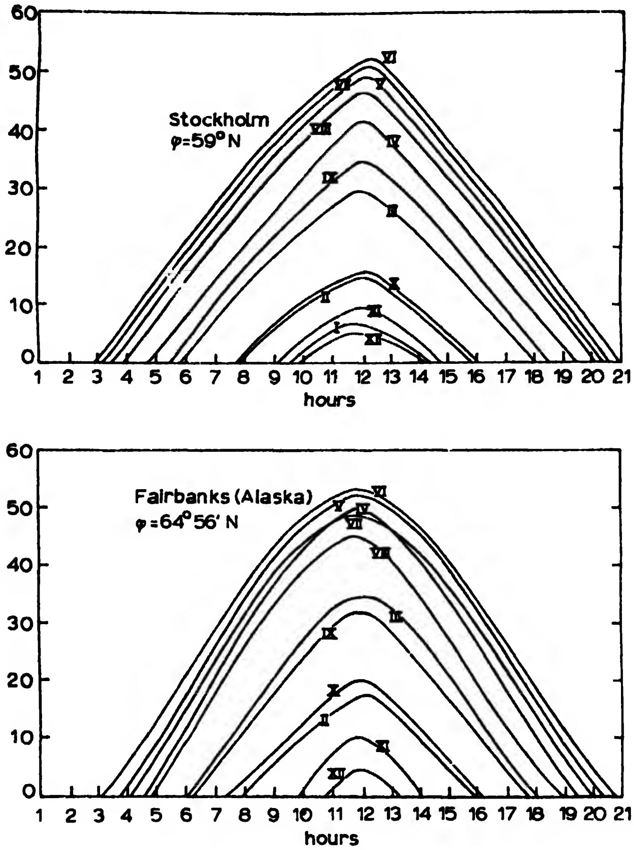

Fig. 5. Hourly solar radiation in Stockholm and Fairbanks ($cal/h \cdot cm^2$).

IGY also show that the radiation charts published by Black in 1956 and by Budyko in 1955 completely neglected the great intensity of summer radiation in the far polar regions. This intensity seems to have a significant influence on thermal conditions both on land and sea.

Comparison between radiation in the Alps and in the subtropics is of great interest too. The radiation observed at altitudes of 1600 m and 3000 m (Davos and Weissfluhjoch) is stronger than any ever observed in the lowlands and approaches subtropical values.

Hitherto, we have discussed caloric radiation, including the infrared and the visible spectrum, to the exclusion of the shortwave ultraviolet radiation. The infrared takes up about 65% of the total spectrum. In addition, the visible part of the spectrum is observed separately, in units of illumination.

Light

Light intensity is measured by means of a selenium photo cell. About forty years ago, Carl Dorno introduced observations by means of the Eder-Hecht "graukeil

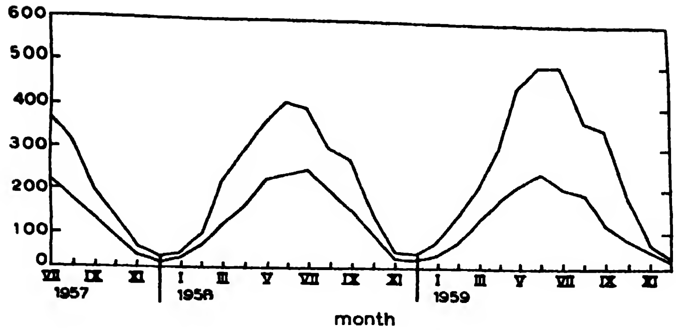

Fig. 6A. Daily global and diffuse solar radiation (cal/cm²·day). Station: Uccle, Belgium, lat. 50°48′ N., long.: 4°21′ E., H (m): 100.

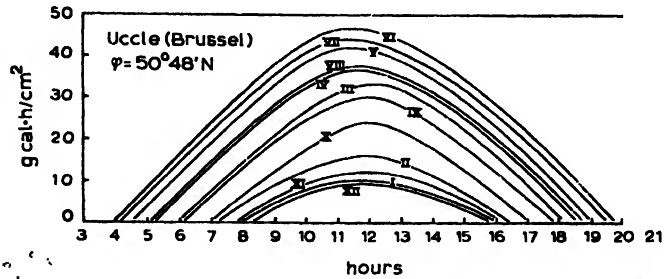

Fig. 6B. Hourly global solar radiation in Uccle (cal/h·cm²).

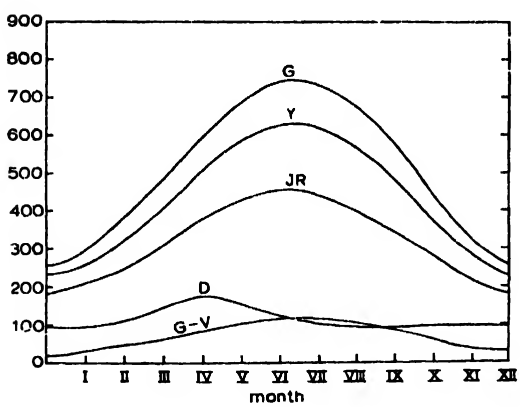

Fig. 7. Annual distribution of global and diffuse solar radiation, and spectral subdivision. Y — yellow filter OG_1; JR = red filter RG_2; D — diffuse radiation; G–Y = global minus yellow filter (corrected); all values in cal/cm²·day.

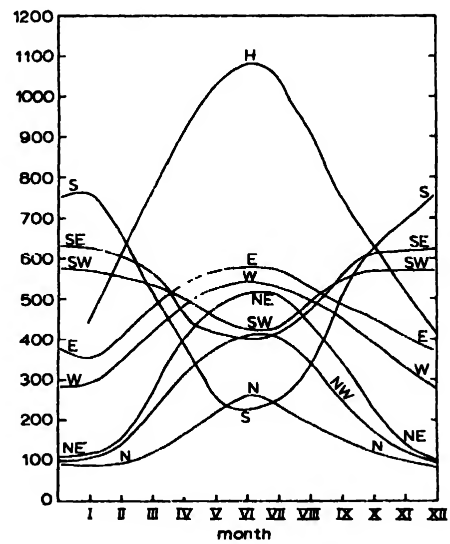

Fig. 8. Jerusalem, daily light amount on different walls (klux·h/day).

photometer" in various places throughout the world. However, this method of measurement has been discontinued. Today light measurements are conducted in only a very few places. In the temperate zones, observations were taken during last years at four stations in England and a few stations in the United States. Observations were also conducted in Vienna and a few other places in Central Europe. In Fig. 11 we see a comparison of the yearly variation of light intensities at Jerusalem,

References p. 101

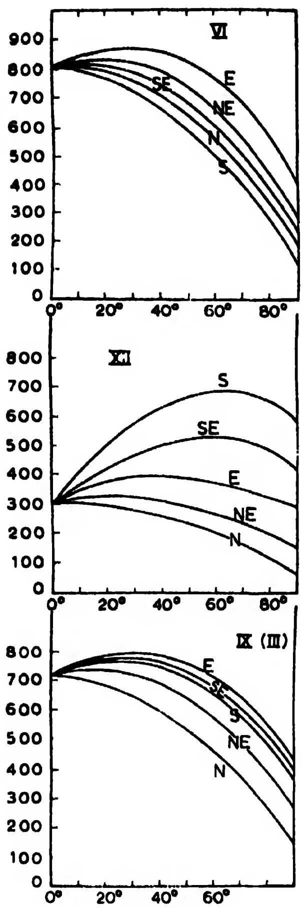

Fig. 9A. Solar radiation in Jerusalem on different slopes in June, December, March and September.

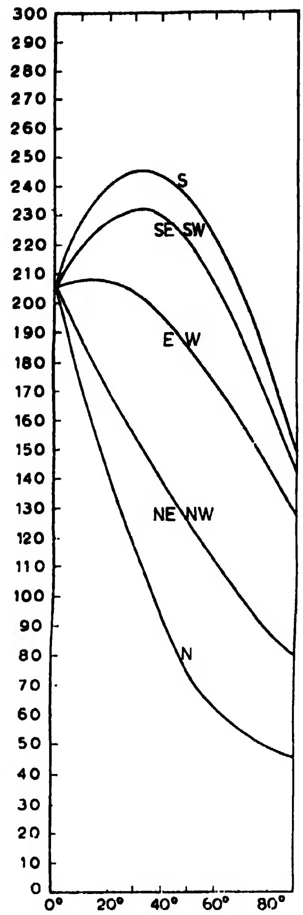

Fig. 9B. Annual solar radiation on different slopes of 8 major directions in Jerusalem (cal/cm²·year).

Vienna and Kew. It is apparent that the daily amounts of light at latitudes 30°–33°N in winter are equal to the summer values of 53°N, while the amount of light measured in the subtropical summer is $2^1/_2$ times greater than at 50°N.

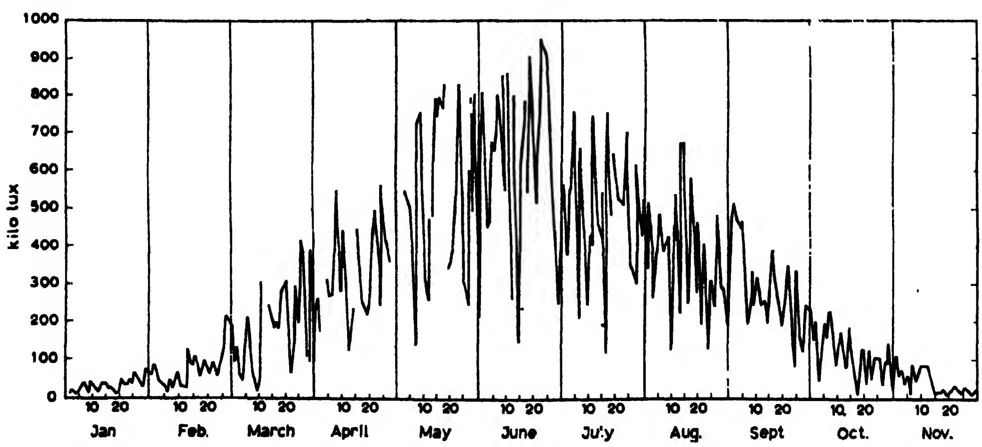

Fig. 10. Daily light in Lerwick.

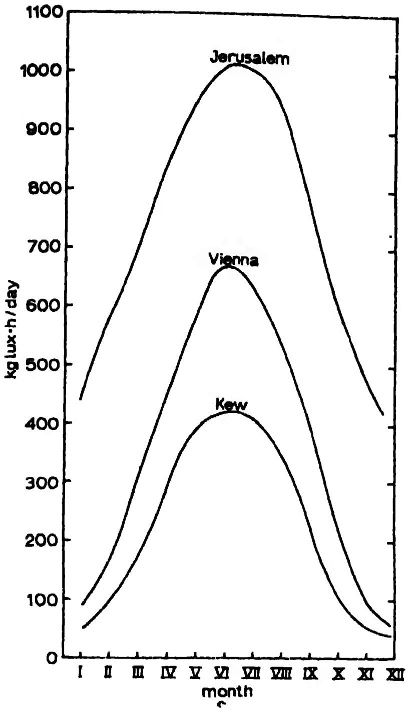

Fig. 11. Light per day (klux·h).

	Kew	*Vienna*	*Jerusalem*
I	045	083	453
II	095	161	555
III	185	304	716
IV	300	468	853
V	400	611	987
VI	425	678	1038
VII	417	658	1016
VIII	350	538	964
IX	235	396	924
X	116	229	664
XI	055	097	509
XII	040	060	430

We have computed the daily and monthly intensities of radiation and light incident upon unit areas, oriented in various directions and tilted at various angles between the horizontal and the vertical. These values are of great biological importance.

Figs. 8 and 9 stress the biological importance of light and solar radiation distribution on walls of buildings and on natural slopes.

REFERENCES

1 D. A. De Vries, *Solar Radiation at Wageningen*, H. Veenmer en Zoon, 1955.
2 D. A. De Vries, *Hourly Observations of Radiation at De Bilt during the IGY* (manuscript).
3 J. Borema and H. P. Berlage, *Solar Radiation in the Netherlands Indies*, 1915-1941.
4 D. Ashbel, *Solar Radiation*, Hebrew University Press, Jerusalem, [illegible].
5 D. Ashbel, *Global Solar Radiation and Daylight in Jerusalem*, Vol. I–III, Department of Meteorology and Climatology, Hebrew University, Jerusalem, 1955, 1958, 1960.
6 D. Ashbel, *Sun's Ray's Intensity in Jerusalem*, Vol. I, 1937; Vol. II, 1942.
7 Meteorological Office, London, *Monthly Weather Report*, Solar Radiation and Intensity of Daylight, 1948-1959.
8 Meteorological Office, London, *Daily Values of Radiation and Illumination in Great Britain* (manuscript).
9 F. Steinhauser, O. Eckel, and F. Sauberer, *Klima und Bioklima in Wien 1955*; South Africa Weather Bureau, *Report*, 1950-55.
10 W.M.O., *Quarterly Radiation Bulletin*, South Africa, 1955-57.
11 H. Lunelund, *Radiation in Finland.*
12 S. Fritz and T. Macdonald, *Some Normal Incidence Solar Radiation Observations during IGY*, 1959.
13 S. Fritz, *Solar Radiation Measurements in Arctic Ocean, Polar, Atm. Symp.*, Part I, Pergamon Press, London, 1958.
14 T. E. Auren, *Studies of Solar Radiation*, Stockholm, 1951.
15 Lindholm, *Globalstrahlung in Stockholm*, 1958.
16 J. N. Black, *Arch. Meteorol. Geophys. u. Bioklimatol., Ser. B*, 7 (1956).
17 Budyko, *Heat Balance*, Moscow, 1955 (in Russian).
18 Jean Dubief, *Le Climat de Sahara*, Tome I, Algiers, 1959, University of Algiers.
19 Solar Radiation in the Congo, *Bull. Mens. Service Meteorol. Congo Belge*, 1952–56.
20 K. J. Hanson, *J. Geophys. Research*, 65, No. 3 (1960).
21 S. Fritz and T. Macdonald, *Heating and Ventilating*, (1949).
22 U.S. Weather Bureau, *Monthly National Summary*, 1950–1959.

SEASONAL VARIATION IN THE ULTRAVIOLET AND INFRARED RADIATION FROM SUN AND SKY AT COPENHAGEN*

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Continuous recording of the amount of ultraviolet sun and sky-radiation in the range 3,100–4,000 Å falling on a horizontal surface element was started in February 1955. From May 1956 another measuring apparatus registering solar and sky radiation in the 2,900–3,100 Å range. For the last year a third apparatus measuring infrared radiation in the range 7,500–11,000 Å has been working. All three recorders are of

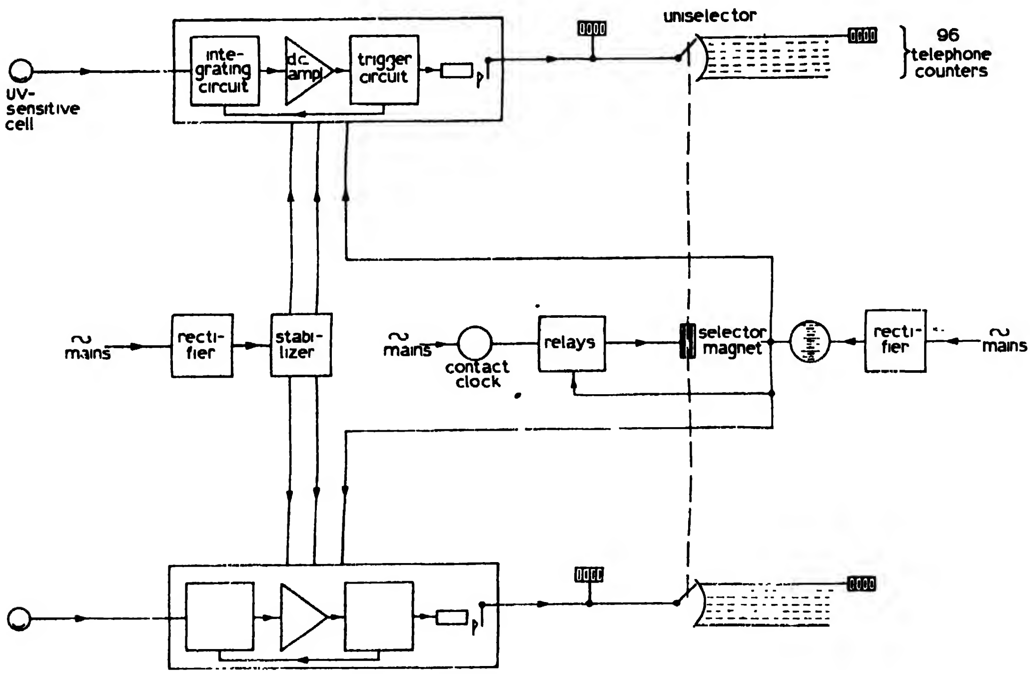

Fig. 1. Measuring apparatus.

similar design, and as the measuring equipment has been described in a previous paper, I shall not repeat the details but only show a block diagram (Fig. 1).

Each of the three recording units is made up of a photoelectric cell provided with a filter connected by coaxial cable to the click-machine, consisting mainly of an integrating circuit followed by a D.C. amplifier and a trigger circuit. By means of a relay this will send impulses both to a total counter and via a pair of uniselectors to one of 96 other telephone-counters. A new counter is switched on every quarter of an hour. The diagram shows only two of the recording units and the main voltage supply.

* Aided by a grant from Statens Almindelige Videnskabsfond and the Finsen Memorial Hospital.

While the two first units are provided with ultraviolet-sensitive cells UNG 7, the third one is furnished with an infrared-sensitive cell (Vacuum type) R.C.A. number 917 and a corresponding filter O.X.5 manufactured by Chance Brothers.

For practical reasons the integrating circuit has been altered so that dead time has been eliminated (Fig. 2).

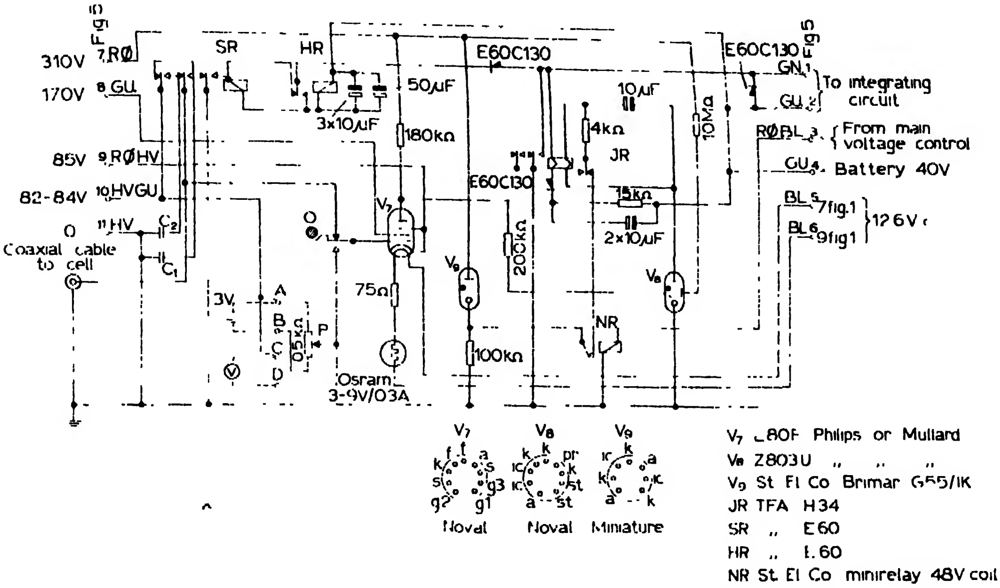

Fig. 2. Measuring apparatus with modified integrating circuit.

Instead of one, there are now two condensers, one — C.1. — is being charged to the normal voltage, while the other — C.2. — is connected to the photocell. With every click, a fast working relay changes the condenser's position in the circuit.

Fig. 3 shows the total ultraviolet energy registered each month. The measurements have been carried out for 5 years, beginning in March 1955. The total number of counts for that month was 140,000 but nearly three times as much was registered in April. In May and June there was still a significant increase, and the maximum value of 721,000 counts occurred in July. In the following months — August, September, October and November the values decreased, the minimum value of 46,000 counts occurring in December.

The diagrams for the following years show nearly the same pattern. Maximum values occur in June or July, minimum values in December or January. From these figures it is easily deduced that most of the ultraviolet radiation received at this geographical location occurs from March to October.

Fig. 4 shows the same measurements in another form and the great difference between summer and winter is more clearly demonstrated. In 1959 the sky was clear most of the time from March to November. The increase from March to June and the decrease from August to the beginning of November was nearly uniform. The maximum value of 1 million counts which occurred in June was the biggest ever registered. The tendency for this year seems to be similar but the values for June and July show something quite different.

In June 1956 the recording of U.V. sun and sky radiation from 2,900 3,100 Å was commenced (Fig. 5). A month by month record over 4 years is shown. In this figure

the minimum of approximately 5,000 counts is found in December, while maximum values of 45,000- 119,000 counts occur in June or July.

Fig. 6 shows a record of infrared in the region 7,500–11,000 Å from two separate days: June 22nd and December 11th, 1959. In June the radiation started at 3 a.m.,

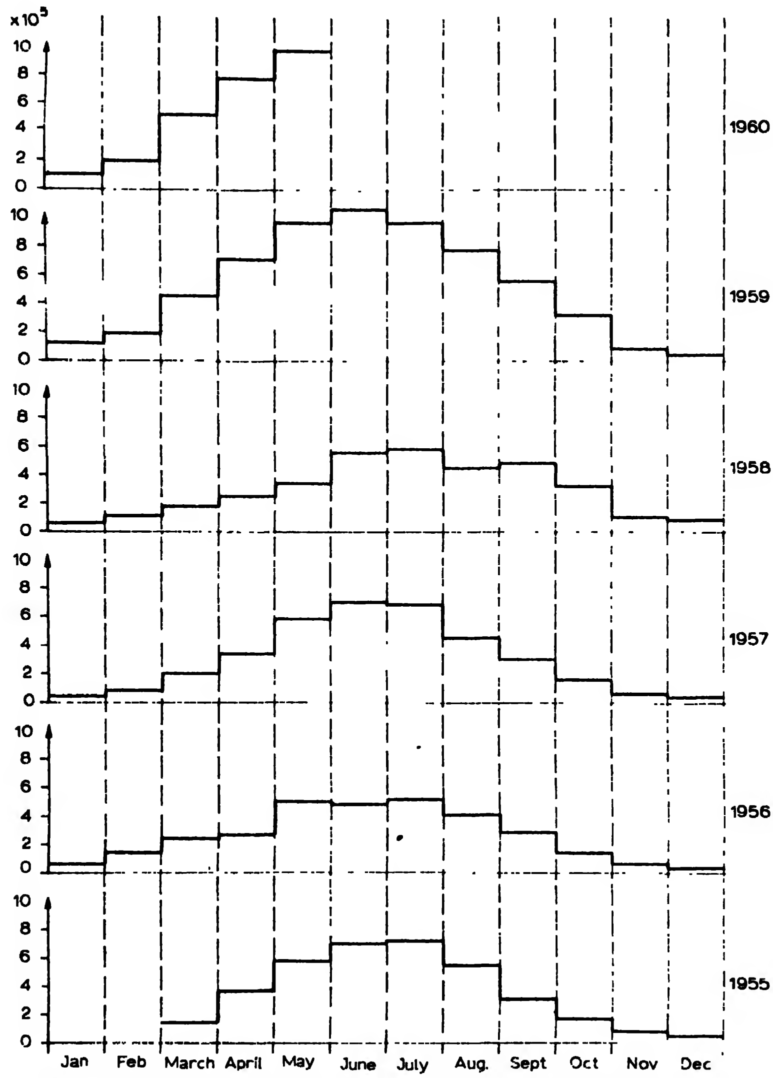

Fig. 3. Total ultraviolet energy recorded from month to month.

and until 9 a.m. there was only a moderate increase from one quarter of an hour to the next. From this moment the values went up very steeply until noon. 1,529 counts were registered between noon and 12.15. Then came the decline: at first very slowly (first two quarters of an hour) and thereafter a regular fall from one 15-min period to the next, until 17.30. Zero was reached at 21.15. The total number of counts for the day was 35,836. On December 11th the infrared started at 8–8.15 a.m. and zero was reached at 16.15. The maximum value for a single quarter hour was 18 counts, the total number was 330.

A month by month record of infrared radiation from June 1st, 1959 to the end of May 1960 is shown in Fig. 7.

The total number of counts for June was 904,000; in July the values were slightly lower. Then followed an almost regular decline until November and in December minimum counts were registered. January this year was a great deal better but February shows twice as much as January. From February to March we have the steepest increase and in May the maximum value of 930,000 counts was obtained.

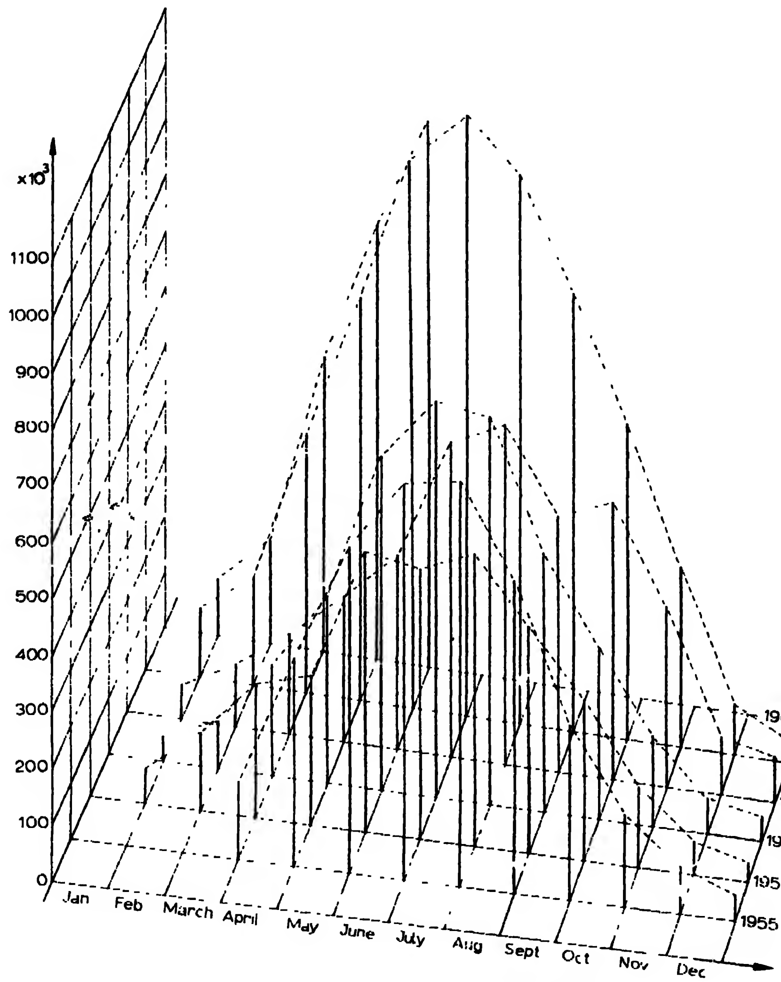

Fig. 4. Total ultraviolet energy.

These measurements cannot be looked upon as typical for Copenhagen, as the weather during the period was far better than normal. It is out intention to continue the measurements for some years in order to obtain mean values.

Fig. 8 shows a daily record of the total counts for our selected wavelength ranges. The abscissa represent the dates, the ordinates are arbitrary units for the three different spectral bands, the top one representing the range 2,900 4,000 Å, the middle one 2,900–3,100 Å and the lower one 7,500–11,000 Å. The measurements cover the period June 1st, 1959 to the end of May 1960. It can clearly be seen that the daily variations generally correspond for the 3 spectral bands. The relative increase or decrease from one day to another in the two U.V. ranges are similar, while the corresponding variations in the infrared band are more pronounced. For example on May 18th, the total U.V. radiation registered was 11,000 counts, the following day shows

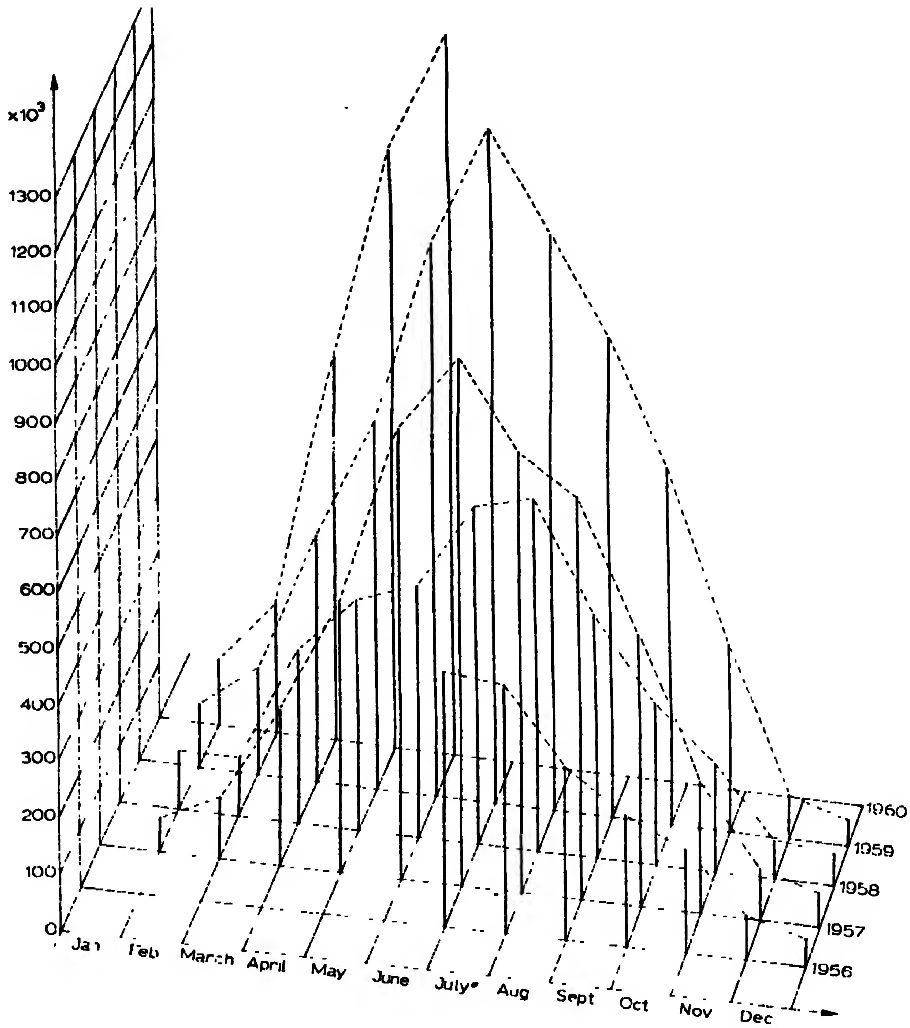

Fig. 5 Record of U.V. sun and sky radiation (2,900 3,100 Å) over 4 years.

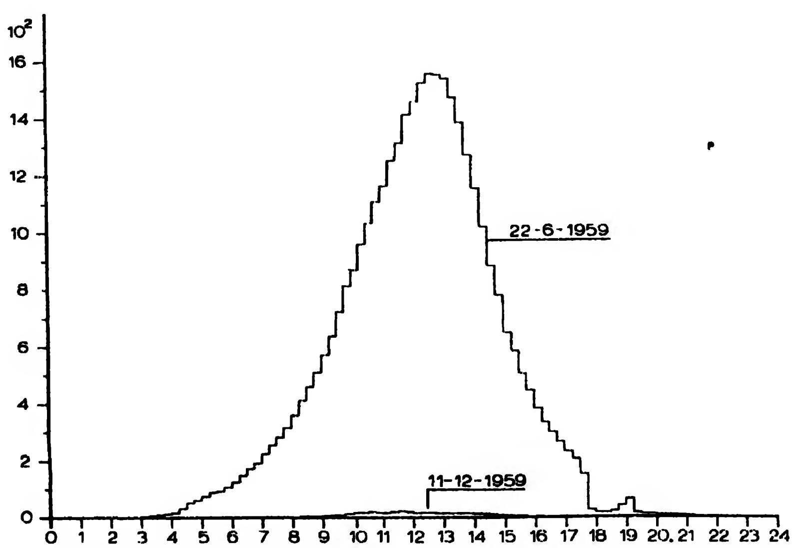

Fig. 6. Record of infrared (7,500-11,000 Å) from two separate days.

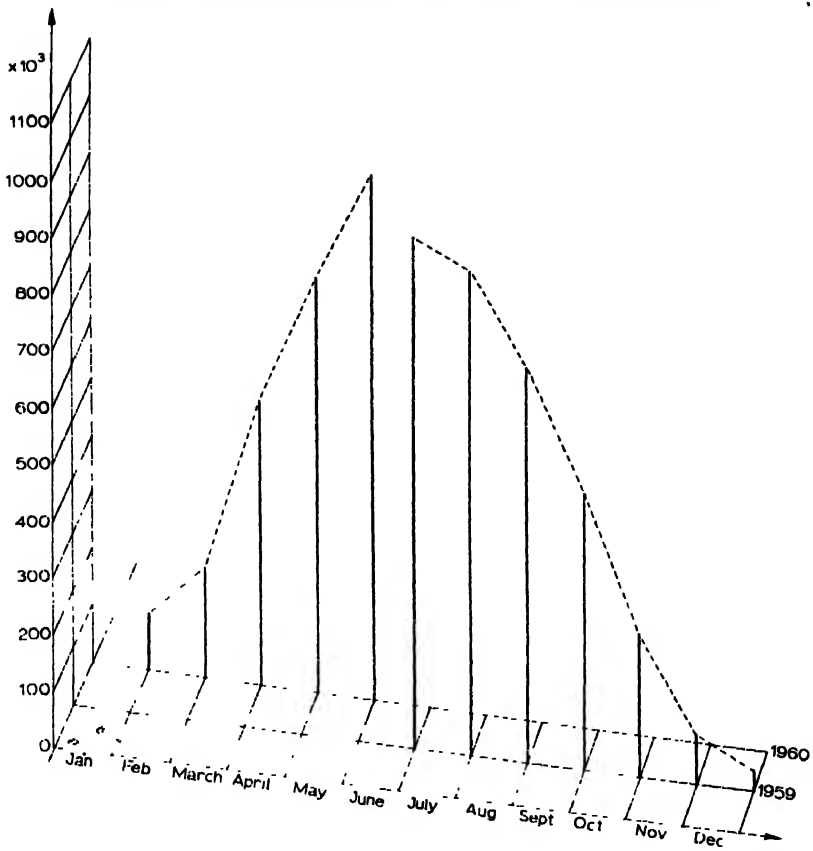

Fig. 7. Month by month record of infrared radiation from June 1st, 1959 to May 31st, 1960.

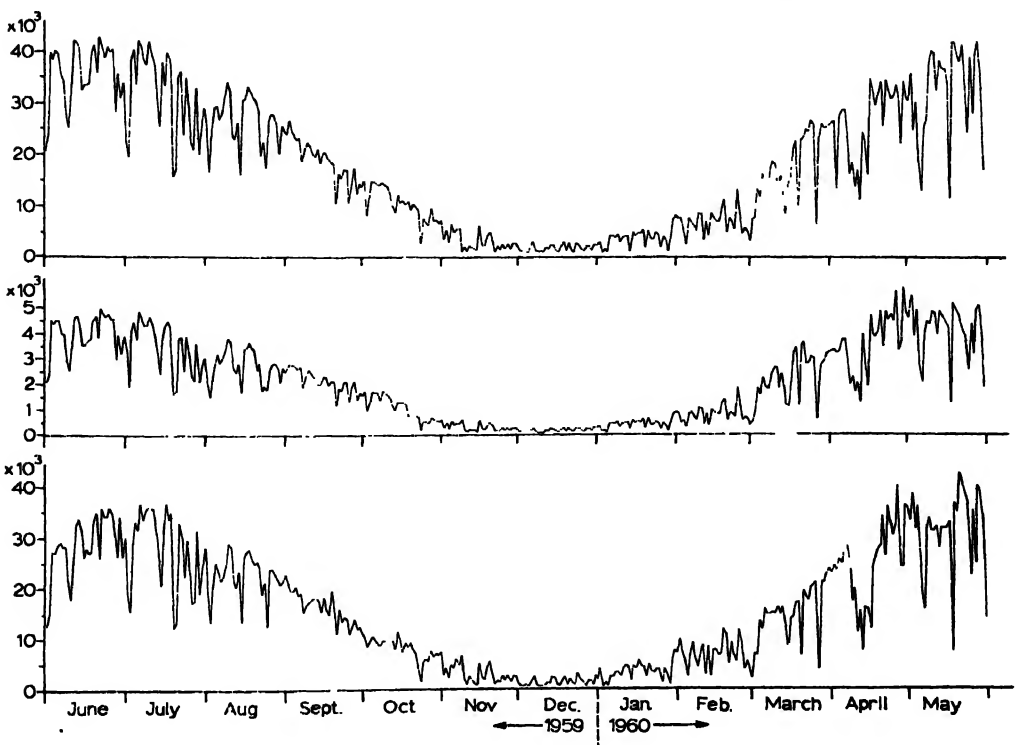

Fig. 8. Day by day record over the same period as Fig. 7.

42,000 counts. The corresponding values in the 2,900–3,100 Å range were 1,200 and 4,200, while the infrared values were 7,500 and 36,500.

These figures show that the really bad weather in May reduced the radiation in the infrared spectral band to 20%, while the U.V. radiation was at the same time only reduced to 26%–28%.

On December 9th the lowest values in all three spectral bands were registered. The total U.V. was 498 counts, the value for 2,900–3,100 Å was 55 counts and the infrared was 319 counts. The highest figures were obtained on June 22nd, 1959, and were 42,587; 4,951 and 35,836 respectively.

Fig. 9 shows the maximum values recorded for a single quarter of an hour out of each 24 hours during the same period and for the same spectral bands. The variations are much less pronounced than in the total daily values.

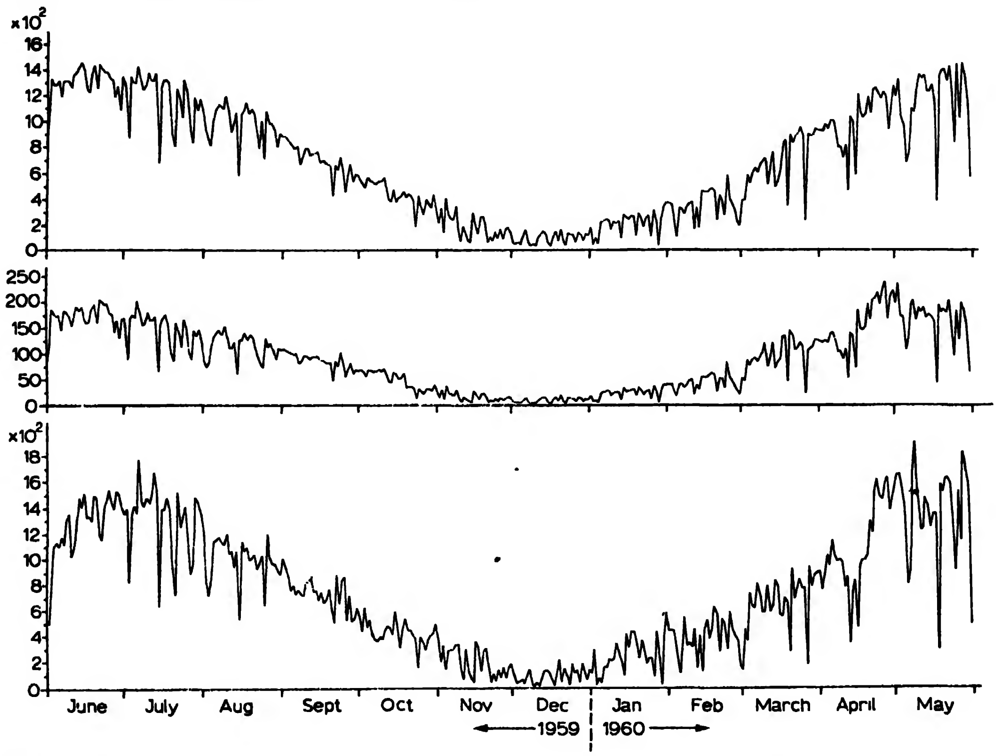

Fig. 9. Maximum recorded values for a single quarter of an hour out of each 24 hours.

The National Bureau of Standards in Washington D.C. was so kind as to carry out the arduous task of adjusting a reference mercury quartz burner for each line. The output of the burner is constant through 100 working hours provided that the burner is kept at a constant level. It was, indeed, a great advantage to be able to check the performance of the entire equipment. Monthly checks reveal no change in the sensitivity. All measurements are given in arbitrary units accurate to within 2%.

Our aim in carrying out these measurements over a number of years and in continuing them, is to obtain real mean values and especially maximum values, *i.e.* the values to which the human organism is exposed.

Later, I hope to be able to carry out similar measurements on the different artificial light sources used in therapy. By comparing these measurements with those for natural light, it may be possible to develop a dosimetry.

VARIATION SPECTRALE ET RÉPARTITION ANGULAIRE DU RAYONNEMENT ULTRAVIOLET DU CIEL

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Un programme d'étude de la luminance ultraviolette du ciel a été entrepris au laboratoire de Physique du Muséum dans le double but d'obtenir par temps très clair des résultats permettant de contrôler les calculs théoriques, et de mettre en évidence les modifications apportées par la pollution atmosphérique, la brume, les différents types de nuages.

En effet la région ultraviolette du spectre semble spécialement avantageuse pour la vérification des résultats théoriques concernant la diffusion Rayleigh, puisque le rôle des diffusions multiples qui constitue le point délicat de la théorie devient de plus en plus important vers les courtes longueurs d'onde. D'autre part l'étude des variations du rayonnement ultraviolet suivant les conditions météorologiques présente à la fois un intérêt pratique évident pour l'utilisation thérapeutique de ce rayonnement et une nette utilité pour orienter des études théoriques tenant compte de la diffusion par les aérosols; la connaissance de ces variations doit permettre aussi d'appliquer des corrections plus précises aux dosages d'ozone faits par spectrophotométrie du ciel zénithal couvert.

ÉTUDE DE LA RÉPARTITION SPECTRALE ET DE LA POLARISATION DE LA LUMIÈRE ZÉNITHALE

L'appareil destiné à cette étude est constitué essentiellement par un monochromateur et un récepteur photoélectrique installés dans un caisson en plein air et par un enregistreur et des dispositifs de commande installés au laboratoire.

La Fig. 1 montre le schéma optique de l'appareil: la lumière arrivant verticalement (dans un angle de 2° environ) par le hublot H est coudée et concentrée sur la fente f_1 du monochromateur Mn, du type à déviation constante, avec prisme de quartz et miroirs aluminiés; la dispersion est de l'ordre de 40 Å dans le violet et 15 Å vers l'extrémité du spectre ultraviolet solaire; malheureusement la lumière parasite bien que réduite par l'emploi d'un filtre ultraviolet Corning dans le faisceau incident (F), devient trop importante à partir de 3200 Å pour permettre de travailler aux plus courtes longueurs d'onde.

La lumière sortant du monochromateur par la fente f_2 est reçue sur la photocathode du photomultiplicateur PM, du type Lallemand à 20 étages et photocathode césium–antimoine. Le courant photoélectrique est enregistré au laboratoire avec un millivoltmètre–potentiomètre Speedomax. Quand ce courant photoélectrique devient trop fort ou trop faible pour permettre un bon enregistrement, des filtres gris consti-

tués par des dépôts de nickel–chrome sur quartz, plus ou moins absorbants, sont automatiquement mis en place dans le faisceau incident (en *G* sur la Fig. 1).

Le tambour qui commande la rotation du prisme, donc le défilement des longueurs d'onde, est entraîné par un moteur électrique à 2 sens de rotation; le balayage complet du spectre ultraviolet dure 8 minutes; le moteur peut être commandé depuis le

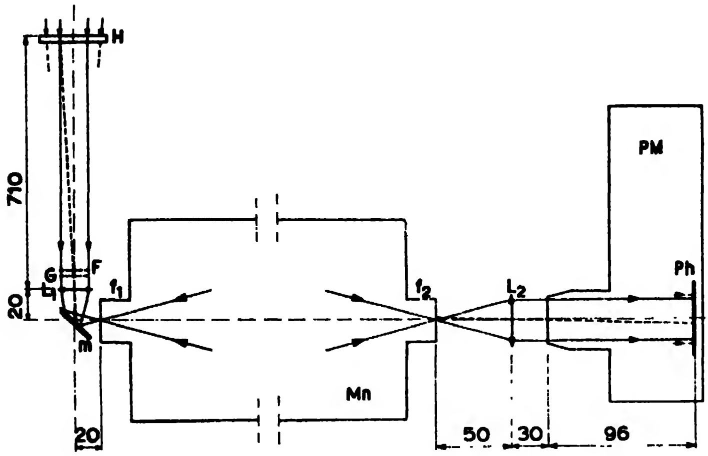

Fig. 1. Schéma optique du spectrophotomètre. *H*, hublot de diamètre30 mm; *F*, filtre ultraviolet;*G*, filtres gris; L_1, lentille de diamètre 8 mm, longueur focale 40 mm; *m*, miroir plan; f_1, fente d'entrée de 0.2 × 1.2 mm; f_2, fente de sortie de 0.2 × 1.2 mm; *Mn*, monochromateur "Géorgie"; L_2, lentille de diamètre 12 mm, longueur focale 50 mm; *PM*, photomultiplicateur; *Ph*, photocathode.

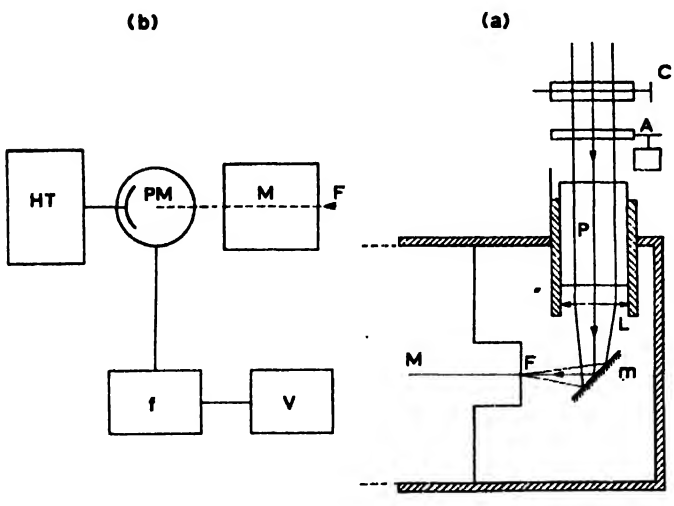

Fig. 2. Schéma de principe du polarimètre. *(a)* partie optique; *C*, compensatrice; *A*, lame demi-onde tournante; *P*, polariseur; *L*, lentille de quartz; *m*, miroir; *F*, fente d'entrée du monochromateur; *(b)* partie électronique; *PM*, photomultiplicateur; *HT*, haute tension; *f*, filtre électrique; *V*, millivoltmètre.

laboratoire, soit à la main, soit automatiquement par des cames entraînées par une pendule électrique, ce qui permet d'enregistrer des spectres à des heures précises choisies d'avance.

Actuellement nous enregistrons des spectres du ciel zénithal aux heures qui correspondent à des hauteurs du soleil de 5°, 10°, 15°, 20°, 30°, 40°, 50°, 60° au-dessus de l'horizon; entre temps on laisse l'appareil enregistrer d'une façon continue la luminance sur 3400 Å. Des enregistrements ont été obtenus régulièrement depuis un an environ à raison d'une dizaine de jours complets par mois et leur dépouillement est en cours.

Quelques mesures de polarisation ont été également faites, mais seulement par lecture directe. On utilise pour cela un petit polarimètre, normalement éclipsé de côté pendant l'enregistrement, et qu'on met en place dans le faisceau incident. La

Fig. 2 montre, à droite, ce polarimètre. La lame demi-onde en quartz *A* tourne sur elle-même à la vitesse régulière de 500 tours/minute et fait tourner à une vitesse double le plan de polarisation de la lumière qui va traverser le polariseur *P*. La fraction polari-

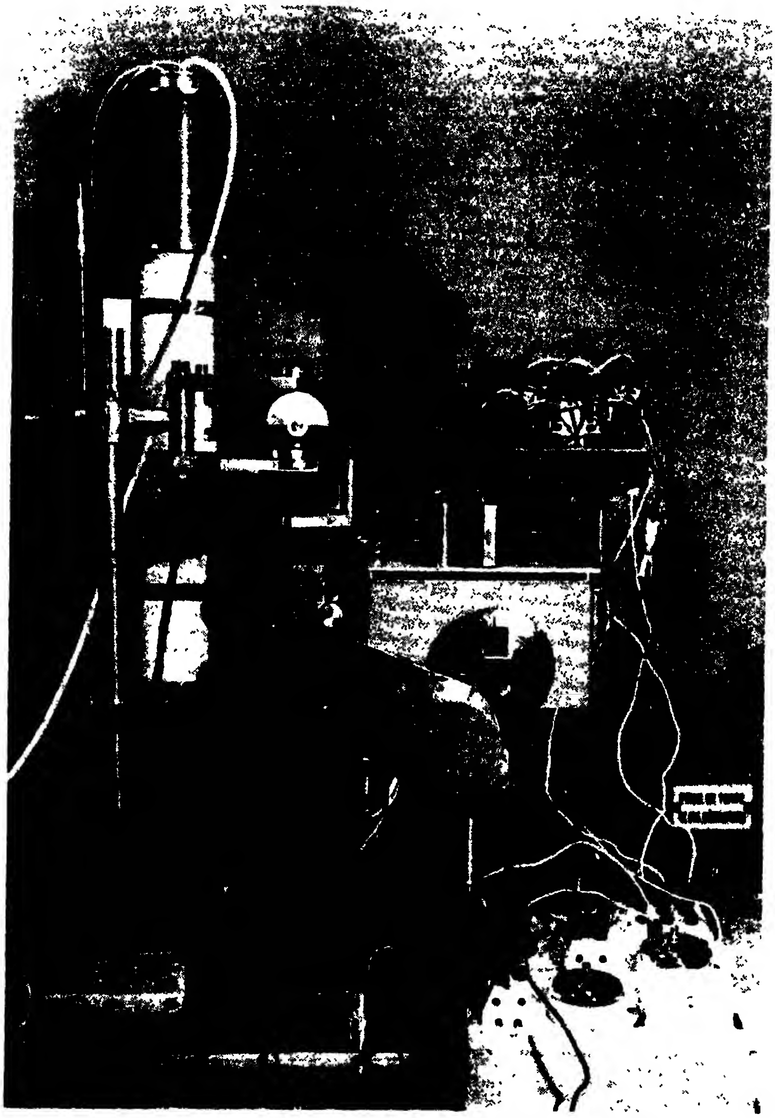

Fig. 3.

sée de la lumière (et le courant photoélectrique qui lui correspond) va donc être modulée à la fréquence de 33 Hz, la lumière naturelle n'étant pas modulée. La partie modulée du courant est séparée par un filtre électrique et lue sur un millivoltmètre électronique (Fig. 2, à gauche).

Au-dessus de la lame demi-onde est placée une lame de silice fondue *C* qui peut tourner autour d'un axe horizontal et de l'axe optique vertical, et qui sert à compenser la polarisation du ciel. Au moment où l'on obtient l'annulation du courant lu sur le millivoltmètre électronique le plan d'incidence sur la lame est confondue avec le plan de polarisation et son inclinaison sur l'horizontal donne le taux de polarisation.

La Fig. 3 montre l'ensemble de l'appareil: monochromateur, photomultiplicateur, moteurs de commande, polarimètre. La Fig. 4 montre le même appareil installé dans son caisson.

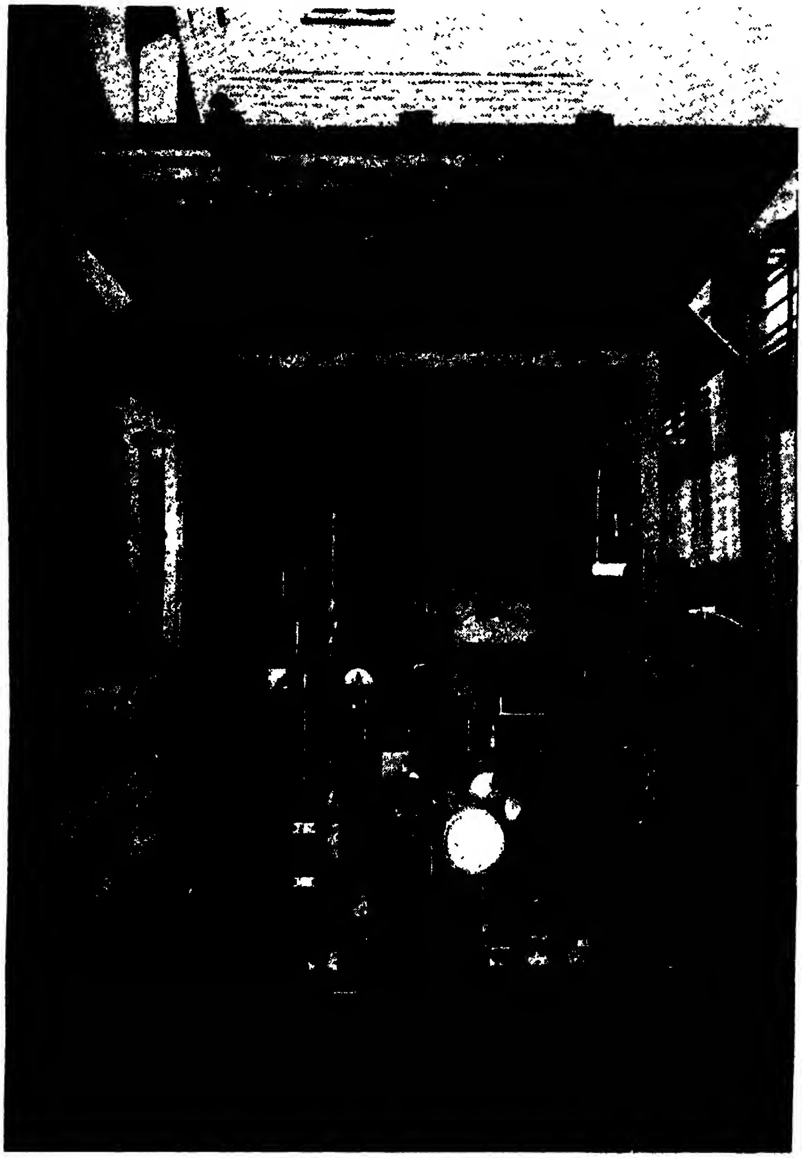

Fig. 4.

ÉTUDE DE LA RÉPARTITION DE LA LUMINANCE SUR LA VOÛTE CÉLESTE

L'appareil destiné à cette étude présente de grandes similitudes avec le précédent. La lumière entrant verticalement dans le caisson (angle de champ de 2° environ) est réduite dans un rapport convenable par des filtres gris et reçue sur la photocathode du photomultiplicateur 20 étages. L'enregistreur et le dispositif de commande des filtres gris sont analogues. Par contre, comme nous nous proposions de n'effectuer le balayage du ciel que sur un certain nombre de longueurs d'onde, nous avons remplacé le mono-

chromateur par une série de filtres interférentiels, ce qui a permis de réduire nettement les dimensions du caisson ; nous n'avons pas non plus actuellement installé de polarimètre dans cet appareil.

La Fig. 5 montre l'appareil, dont trois côtés ont été démontés. La plateforme qu'on

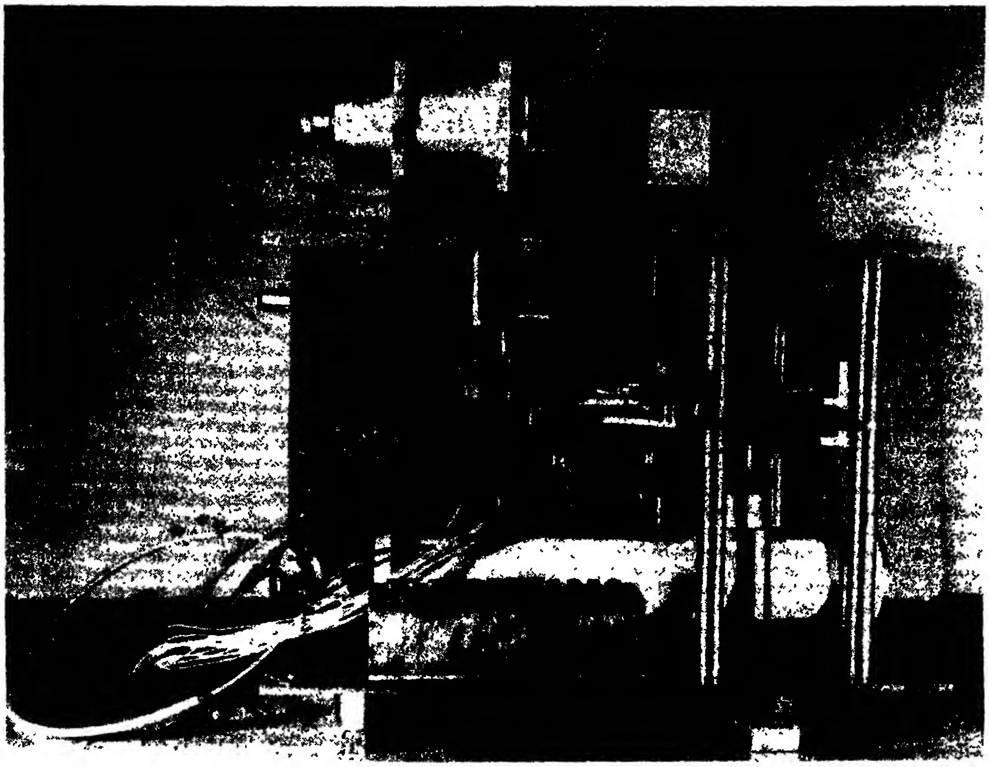

Fig. 5.

voit sur le dessus porte le système de miroirs permettant d'envoyer dans l'appareil la lumière de n'importe quelle direction du ciel; un premier miroir incliné à 45° sur l'horizontal et pouvant tourner autour d'un axe horizontal renvoie la lumière horizontalement sur un deuxième miroir fixé également à 45°, qui la réfléchit verticalement sur le hublot du caisson. La rotation du premier miroir autour de l'axe horizontal permet de balayer toutes les directions d'un plan vertical, tandis que la rotation autour de l'axe vertical de l'ensemble de la plateforme permet de balayer n'importe quel plan vertical. Les deux mouvements sont obtenus par des moteurs électriques commandés depuis le laboratoire.

Cet appareil est actuellement en cours d'essai et doit être mis en fonctionnement prochainement.

Nous espérons que le fonctionnement simultané des deux appareils permettra de réaliser des expériences précisant les résultats donnés par chacun d'eux; par exemple l'utilisation de l'appareil 2 visant au zénith sur une longueur d'onde fixe permettra de bien mettre en évidence avec l'appareil 1 les modifications de la répartition spectrale, indépendamment de toute variation de l'intensité totale. Inversement pendant le balayage du ciel par l'appareil 2, l'appareil 1 pourra enregistrer, à titre de référence, la luminance du zénith sur la même longueur d'onde.

THE MEASUREMENT OF NATURAL ULTRAVIOLET RADIATION

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The study of natural U.V. radiation is of interest to photobiologists from the point of view of its effect on man, agricultural animals, plants and micro-organisms.

The measurement of spectral concentration of irradiance gives the full physical characteristic of natural U.V. radiation $E_{e,\lambda}$[mW/m²] = $f(\lambda)$, where λ is a wavelength[1,2]. The measurement of integral irradiance E or irradiation H is easier in some regions of the U.V. spectrum.

It is often necessary to evaluate natural U.V. radiation according to its erythemal or antirachitic effect. If it is assumed that the action spectra over the range 290–320 mμ are very similar, it is possible to use effective erythemal values — erythemal irradiance E_{er}[er/m²] or erythemal irradiation H_{er}[er.min/m²]*. We recommend the measurement of the values E_{er} and H_{er} both on a plane and on a sphere of small radius ("mean spherical")[4,5].

The apparatuses designed for U.V. climate measurements have to comply with certain special requirements, *viz.* good spectral sensitivity (for integral-type apparatus); reliability of operation over significant changes of temperature and humidity of ambient air; yielding of readings which are proportional to the cosine of the angle of light incidence when measured on a plane; measurement of the "mean spherical" should not lead to readings dependent on the angle of light incidence, etc.

Various models of such equipment were designed by the authors in the National Lighting Institute (NLI) and the Institute of Biophysics of the Academy of Sciences of the USSR (IBPh) in cooperation with D. A. Shklover, V. V. Koltsov, V. A. Ilyanok, I. G. Bukhartsev and others. These are described below. The photoelectric measurement method is used in these sets since this has well known advantages over the other measurement methods.

(*1*) A prototype model has been designed by IBPh for periodical measurements of $E_{e\lambda}$, = $f(\lambda)$. This set consists of a specially designed portable monochromator, Sb-Cs photomultiplier and a recording electronic potentiometer. A replica of a concave diffraction grating (with a focal distance of 0.5 m) is used in an autocollimation-type monochromator; this makes it possible to dispense with focussing devices and thus design a monochromator of small dimensions. Successive exposure of given monochromatic rays is carried out by turning the replica. An optical device containing an integral sphere is placed in front of the entrance slit of the monochromator.

* The erythemal flux means radiant power of U.V. radiation at λ > 280 mμ, which is evaluated according to the erythemal effect of radiation on mans skin. er — Unit of erythemal flux — is equal to the erythemal flux corresponding to one watt of radiant flux at a wavelength 297 mμ[3].

(*2*) The first integral-type set for continuous automatic measurement of U.V. irradiance has been designed by NLI. The set has a Sb-Cs photocell SCV-6, together with light filters for measurements in the region of 290–340 mμ with maximum at about 315 mμ, and an electronic potentiometer with a circular scale. Total and diffuse U.V. irradiance have been studied by means of this set for two years (1957, 1958) in the town of Evpatoriya[6].

The recording set designed by IBPh, in contrast to the previous one, permits measurement of erythemal irradiance [mer/m^2], irradiance in three comparatively narrow regions of the U.V. spectrum [mW/m^2] and illumination [lux] (Fig. 1). This set has

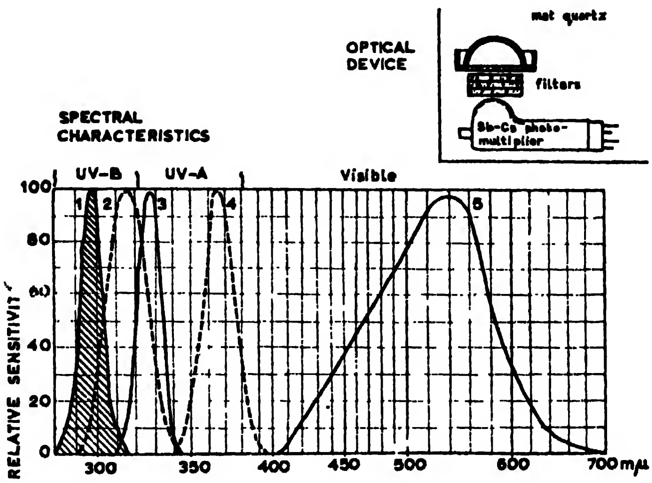

Fig. 1. Recording set for measurement $E_{er} = f(t)$ and other characteristics. 1 — glass and gelatin filter with picric acid; 2–5 — glass filters.

six small-size Sb-Cs photo-multipliers (manufactured by National Electrotechnique Institute) and sets of glass filters. The sixth photo-multiplier provides a zero reading. The current from the photo-multipliers which are activated by dry batteries, is measured by means of a six-point electronic potentiometer EPP-09. This set is at present tested at the Meteorology Observatory of the Moscow State University (Fig. 2).

(*3*) A different portable integral-type apparatus is used for periodical measurement of the U.V. climate in the USSR. A small type of uphimeter UF-I has been put into operation by IBPh[3]. This apparatus contains photocells F-7 with a flat magnesium cathode and hemispherical light filter made of BS-4 glass. The long-wave limit of spectral sensitivity of the apparatus is about 345 mμ (Fig. 3, curves Mg+BS-4).

To measure natural U.V. irradiance the apparatus is calibrated in energy or erythemal units [mW/cm^2 for 290–320 mμ regions or er/m^2]. This type of apparatus, known as UFM-II, is now being manufactured in quantity.

Some experimental uphimeters (Fig. 4) equipped with a spherical photocell with a semi-transparent Sb-Na photo-cathode have been produced to measure mean

References p. 118

spherical irradiance. The photocells were designed by T.N. Rabotnova and N. N. Gortchakova[7]. The photocell spectral sensitivity is shown in Fig. 3.

The uphidosimeter UFM-5 (designed by NLI) and uphidosimeter UF-2 (designed by IBPh) are often used for *II* measurement. The first apparatus has an Sb-Cs photocell, light filters and impulse counter[8]. The spectral sensitivity curve of the apparatus is the same as the one in Fig. 1, (curve 3).

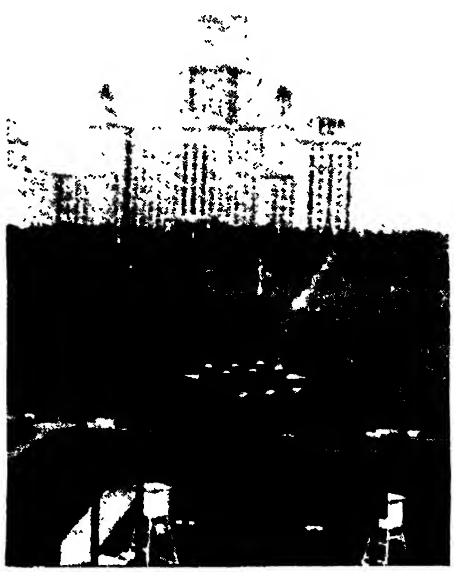

Fig. 2. Placing of recording set.

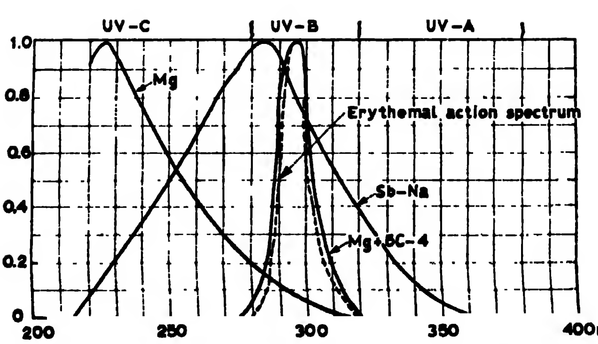

Fig. 3. Spectral sensitivity of certain apparatuses for measurement of U.V.-radiation.

The uphidosimeter UF-2 has a magnesium photocell F-7 and a hemispherical light filter made of BS-4 glass which closes a photocell when measurements are being made in the spectral region of 280–320 mμ. The spectral sensitivity in this case approaches the spectrum of the erythemal effect (Fig. 3, curve Mg+BS-4). The electrical part of

the apparatus is based on a sensitive bridge circuit (Fig. 5), and the radiation measurement is made by impulse counter. The apparatus is also provided with sound signalling[9].

Fig. 4. Uphimeter for measurement of mean spherical irradiance

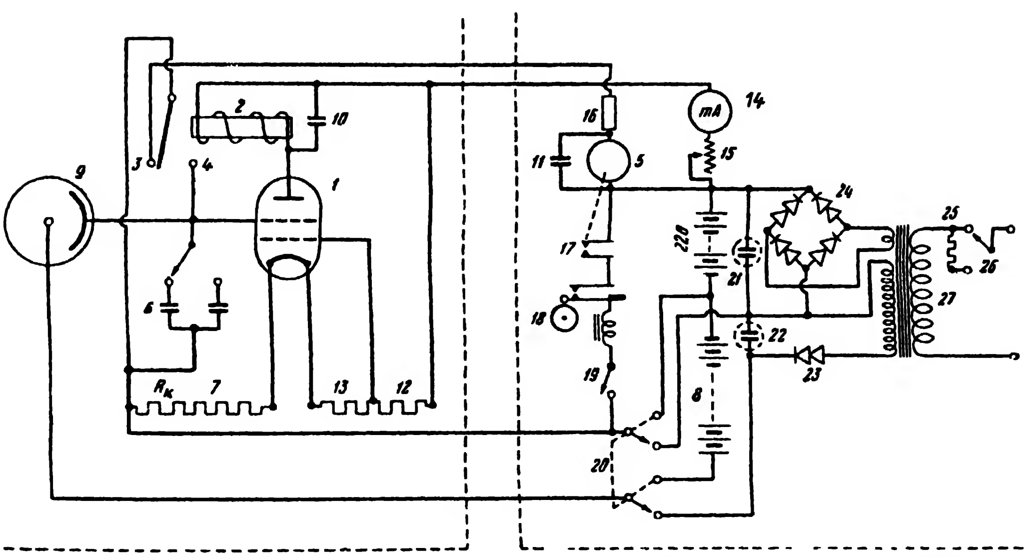

Fig. 5. Electrical scheme of uphidosimeter UF-2: 1. electrometric tube 1E1P; 2. polarized relay PRP-4; 3. relay contact; 4. normally opened relay contact. 5. counter of SB-I type; 6. scaling condensers; 7. 250 Ω resistance; 8. battery GB-100; 9. magnesium photocell; 10. condenser 1000 pF; 11. condenser 1000 pF; 12,13. wire resistance of 55 Ω each; 14. milliammeter M-62 with scale for 100 mA; 15. filament resistor (150 Ω) with switch; 16. 2 kΩ resistance; 17. counter contacts closing the bell; 18. bell; 19. bell switch; 20. external and internal supply switch; 21. condenser 100 μF; 22. condenser 50 μF; 23,24. selenium rectifier, 25. resistance 2.5 kΩ; 26. 127–220 V switch; 27. step-down transformer.

References p. 118

Calibration of each type of apparatus was carried out by the same method, *viz.* using U.V. standard lamps, erythemal fluorescent lamps EUV-15 and band incandescent lamps SI-16 with a U.V. glass window.

CONCLUSIONS

It is evidently necessary to standardise the methods of measurement of natural U.V. radiation. In this connection the authors suggest the following propositions:

(*1*) Study of the U.V. climate in order to record periodically the spectral concentration of irradiance and to continuously record erythemal irradiance on a horizontal plane;

(*2*) Dosimetry studies of the solar bath to measure mean spherical erythemal irradiation.

REFERENCES

1 R. Herrman, *Optik*, B.2 (1947) 384.
2 R. Schulze, *XII. Session CIE*, v.11, 1951.
3 D. N. Lazarev, M. V. Sokolov and D. A. Shklover, *XIV. Session CIE*, P.59.3, 1959.
4 M. Bender and F. Krüger, *Phys. Z.*, 35 (1934) 321.
5 K. Bunter and E. Suffer, *Strahlentherapie*, 45 (1932) 445.
6 O. P. Shelkova-Dorf, D. A. Shklover and I. T. Jakovleva, *Svetotekhnika*, No. II (1958) 21.
7 T. N. Rabotnova and L. V. Konontchuk, *Svetotekhnika*, No. 9 (1959) 19.
8 D. A. Shklover and O. P. Dorf, *Svetotekhnika*, No. 3, (1956) 20.
9 A. P. Andreitsev and P. A. Osetrov, *Information Bulletin of VINITI, Moscow* (1957), theme 37, No. II-57-44/2.

Symposium 2

BIOLOGICAL ACTION SPECTRA*

Chairman: K. V. THIMANN, Cambridge, Mass. (U.S.A.)

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ACTION SPECTRA OF PHOTOTAXIS IN UNICELLULAR ALGAE*

PER HALLDAL

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ACTION SPECTRA IN THE VISIBLE REGION

Phototaxis involves locomotive movements in which organisms or cell fractions are attracted (positive phototaxis) or repelled (negative phototaxis) by light. In the flagellates it is assumed, but not proved, that the photoreceptor for phototactic response is sited near the flagellar base. It is also generally agreed that the stigma or the so-called eyespot is not a part of this photoreceptor, and it is certain that the stigma is not needed for photic orientation. Phototaxis has recently been excellently dealth with in articles by Haupt[1] and Bendix[2], where these problems are discussed.

The photic orientation in the flagellates is most reasonably explained by assuming an eccentrically situated photosensitive spot connected to the flagellum through which the light orientation is mediated. This tiny spot, which is only a fraction of a μ in size, does not possess any structure that possibly could detect light direction by a focusing process. Another principle has therefore been applied for light orientation within the flagellates. A pigmented mass within the cell would in some positions cast a shadow upon the photoreceptor. Thus in the visible region two pigmented organs are needed for phototaxis. One of these would be the photoreceptor; the other could be any item which, when inserted in the light path, reduces the light intensity by a certain amount. If Weber's law is valid this reduction must be at least 10% (Halldal[3]).

Some examples will illustrate what is meant by this. It is possible to develop different types of *Euglena* by certain treatments. In this way it has been possible to obtain, in addition to normal *Euglenas* with chloroplasts and stigma, also completely colourless forms, and also forms without chloroplasts but with stigma. As both *in vivo* absorption measurements and action spectra analyses have been performed on these different types, a series of examples is here available which may illustrate the effect of shadow-casting pigmented masses within the cells. If we assume that the absorption characteristics of the pigment involved in photic orientation are not affected by the treatments which produce these different *Euglena* types, the effect of the shadow-casting mass may be studied directly. In Fig. 1 are schematically drawn these three *Euglena* types, their action spectra of phototaxis, and their *in vivo* absorption characteristics. The *in vivo* absorption curves are based upon measurements by Shibata *et al.*[4]. The action spectra measurements were performed by Gössel[5] for the colourless form and for the stigma-containing form without chloroplasts. Bünning and Schneiderhöhn[6] carried out the measurements on normal *Euglena*. The Tübingen group made a distinction between two different types of phototaxis, namely topic and photic (Haupt[1]). As the same pigment seems to be involved in these two reaction types, such a distinction does not have any bearing upon our considerations about a light absorb-

* Invited paper.

References p. 126

ing item (pigment involved in phototaxis) and a shadow-casting mass. It is demon strated by this series that phototactic response only occurs in the spectral region where the cell shows measurable absorption. For the colourless form a sharp maximum appears at 410 mμ, that is, just in the spectral region where the cell starts to absorb.

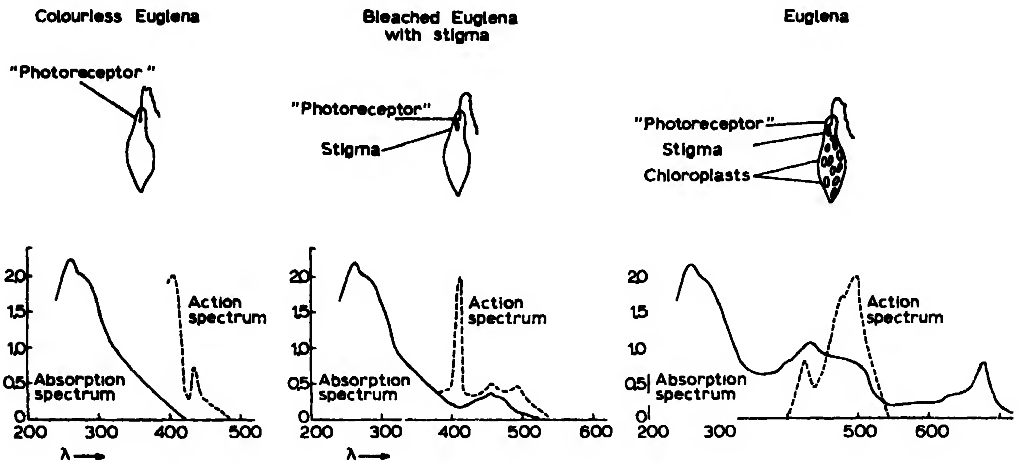

Fig. 1. Action spectra of phototaxis (Gössel[5]: the bleached and colourless form; Bünning and Schneiderhöhn[6]: the normal form) and *in vivo* absorption curves (somewhat modified from Shibata *et al.*[4]) of different forms of *Euglena*.

The method of Shibata *et al.*[4] corrects for selective scattering. If selective scattering is taken into consideration, the violet light that passes through a cell is more reduced than light of longer wavelengths. This will accentuate the "shadow-casting ability" of cells in the Soret region. For the chlorophyll-free form with stigma the effect of blue light is somewhat higher in photic orientation. The most reasonable explanation for this is an effect of a shadow from a pigmented mass which absorbs in this spectral region, namely the stigma. Due to the relatively small size of this pigmented mass, light passing through a cell will on most occasions not hit the stigma in such a way that a shadow can be cast upon the photoreceptor. Thus, only on a few occasions can the photoreceptor receive the necessary signal for adjustments in swimming direction. For the chlorophyll-containing form the pigmented masses within the cell (chloroplasts) absorb sufficient light at all wavelengths to reduce the intensity by the required amount (at least 10%). The action spectrum curve for this type has a maximum at 490 mμ with some minor absorption bands at shorter wavelengths. This is most probably the only action spectrum from *Euglenas* which gives significant information about absorption characteristics of the pigment involved in photic orientation. Action spectra curves with similarities to this have been obtained for several other species. Maxima for species within the *Volvocales*, *Ulva-gametes*, *Dinophyceae* usually occur between 475 and 495 mμ (Mast[7], Halldal[3]). An exception to this was observed in the dinoflagellate *Prorocentrum micans*, where an action spectrum with maximum at 570 mμ was recorded (Halldal[3]).

When action spectra analysis are to be performed for the purpose of getting information about absorption characteristics of pigments involved in phototaxis, it is therefore safe to do the experiments only on coloured forms.

ACTION SPECTRUM OF PHOTOTAXIS IN ULTRAVIOLET REGION

The action spectrum of phototaxis has been determined in the ultraviolet region for the photosynthetic flagellate *Platymonas subcordiformis* (*Volvocales*) by Halldal[8]. The result for the negative response is given in Fig. 2. The same action spectrum curve was obtained for positive phototaxis. The measurements were performed with a

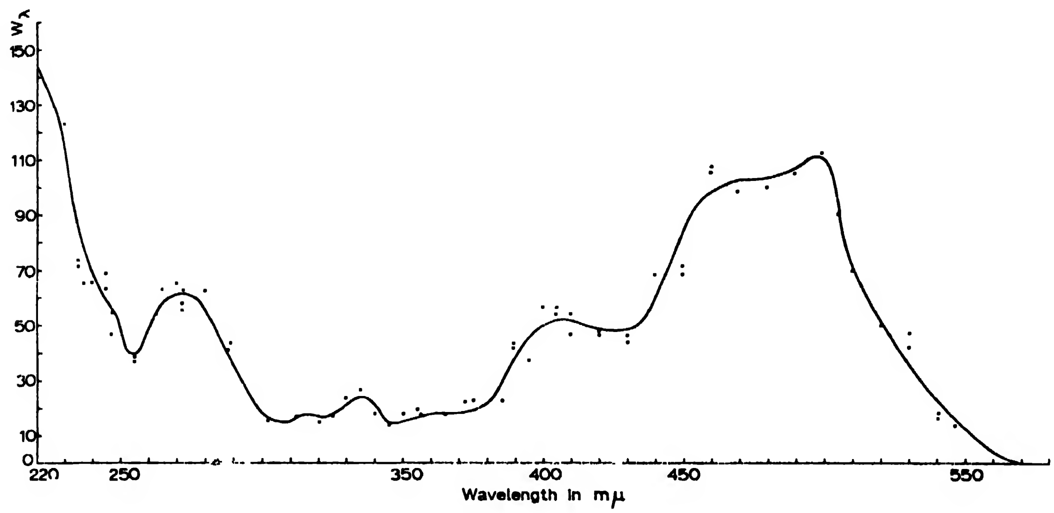

Fig. 2. Ultraviolet action spectrum of negative phototaxis in *Platymonas*.

Bausch and Lomb 250-mm grating monochromator with a mercury-arc light source. Outside the mercury lines spectral bands of 5 mμ were used. The radiant intensity emerging from the monochromator was adjusted by means of the Vee-slide of the exit slit which controls slit length. The sample, contained in a quartz cuvette, was placed directly outside the exit slit at the image of the grating. Radiant intensity was measured with a RCA 1P28 photomultiplier tube. The relative sensitivity curve given by the manufacturer for this tube was used in combination with a Moll and Burger Standard Thermopile and a calibrated Kipp and Sons galvanometer type Pa. The threshold values for phototactic response were determined. The threshold value at 405 mμ for positive phototaxis was 0.15 ergs/cm^2 · sec, and for negative phototaxis 0.80 ergs/cm^2 · sec.

The 220-mμ radiation had a very high phototactic effect. Towards longer wavelengths the activity dropped quickly and a minimum was recorded at 255 mμ. A clear maximum occurred at 275, and a smaller at 335 mμ. In order to get the curve complete the measurements were also repeated for the visible region. A small maximum appeared at 400 to 405 mμ. This was not recorded in earlier experiments by Halldal[3] for this species. However, the measurements around 400 mμ in Halldal's earlier experiments are rather uncertain. This new detail shows a similarity to action spectrum measurements for *Euglena* in this spectral region by Bünning and Schneiderhöhn[6].

Action spectra of phototropism in *Phycomyces* have been determined by Curry and Gruen[9] and by Delbrück and Shropshire[10]. These action spectra and the action spectrum of phototaxis in *Platymonas* show some striking similarities. In *Phycomyces*

References p. 126

phototropism is reversed below 300 mμ. Accepting Delbrück's and Shropshire's explanation of a substance (gallic acid) acting as an internal screen in this spectral region, the peaks, even if the reaction is reversed, may stand for absorption bands of the pigment involved in phototropism. Delbrück and Shropshire observed peaks at 280, 385, 455 and 485 mμ; and Curry and Gruen at 280, 370, 445 and 470 mμ. The minimum at 250 mμ and the increased activity below this wavelength recorded by Curry and Gruen are also similar to the action spectrum of phototaxis in this spectral region. These facts suggest that related pigment complexes are involved in phototropism in *Phycomyces* and phototaxis in flagellates. We do not wish at this time to speculate about the nature of these pigments or their complexes.

ACTION SPECTRA OF INDUCED PHOTOTACTIC RESPONSE

In the salt water green flagellate *Platymonas subcordiformis* (Wille) Hazen (Gibor's strain), a desired phototactic response may be induced at will by adjustments in the ion composition Ca^{++}, Mg^{++} and K^+, provided proper pH and ionic strength are maintained (Halldal[11]). This has made it possible to produce *Platymonas* populations which divide into about 50% negatively and 50% positively reacting cells. For the action spectra analyses which will be presented here about 200 ml of a *Platymonas* culture was centrifuged at 500 *g* for 5 min and resuspended in the following solution: 0.5 *M* NaCl; 0.01 *M* $CaCl_2$; 0.02 *M* $MgCl_2$; 0.005 *M* KCl; 0.001 *M* $KHCO_3$. The pH of this mixture was 7.5. In this solution *Platymonas* showed a 90 to 100% negative reaction, which in the dark was retained for at least 12 h. In the dark the response had changed to positive in 24 h, and a few minutes of "white" light reversed the reaction from negative to positive. The effect of different wavelengths of light was studied in this reversion process (negative to positive and *vice versa*, Halldal[12]). The measurements were performed in the following way: 0.3 ml of the suspension was placed in a 1-cm path Beckman cuvette with a polished bottom. In order to prevent the algae from settling by phototaxis and gravity, the cuvette was rotated around its vertical axis at about 100 revolutions per minute and the sample illuminated from below with "monochromatic" light from a projector constructed for this experiment. A constant intensity of 21,000 ergs/cm^2 · sec was used at all wavelengths. Spectral bands were isolated by means of combinations of interference filters and Schott and celluloid filters. Infrared radiation was removed by $CuSO_4$-liquid filters or water. After a predetermined time the exciting light was shut off, the motor stopped, and the sample illuminated from the side with low intensity blue-green light which accumulated the cells phototactically. The accumulation was studied in a horizontal microscope with weak red light as microscope illumination. The shortest exposure time that caused a complete reversal was recorded. The reciprocal of this time was then plotted against wavelengths of light, and these curves corrected for incident quanta give the action spectra for these transformations.

Induced positive phototaxis

The result of the negative to positive transformation is given in Fig. 3. Violet to green (400 to 540 mμ) and red light (660 and 685 mμ) induced positive response in a population with negative phototaxis. It was not possible to induce positive response between 540 and 655 mμ. At 655 mμ the population divided into about 50% negatively

and 50% positively reacting cells. The pronounced effect at 660 mμ and the short distance between this wavelength and 655 mμ seem unreasonable. However, the 660-mμ filter had an additional band appearing as a shoulder around 680 mμ which could not be removed by glass filters. For this reason the peak of the action spectrum curve in this region should probably be shifted somewhat to longer wavelengths. No measurements have been performed between 685 and 740 mμ and observations in this region may give additional information. No transformation occurred at 740 mμ.

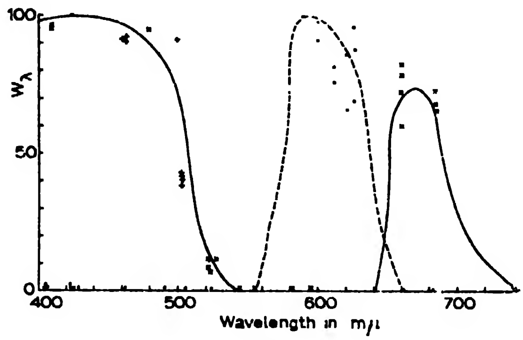

Fig. 3. Action spectra of induced phototactic response changes in *Platymonas*. ——— - - — —: the negative to positive transformation; - - - - - - - -: the positive to negative transformation.

Induced negative phototaxis

A different picture was obtained for the transformation from positive to negative phototaxis (Fig. 3). It was not possible to change a positive population into a negative in the spectral region between 400 and 540 mμ, or at 660, 685 and 740 mμ. A positively reacting population remained positive irrespective of exposure time at these wavelengths. A transformation from positive to negative was obtained between 580 and 630 mμ with a maximum at 590 mμ.

Alternations in response at different wavelengths

A shift in phototactic response occurred when the cells were illuminated at different wavelengths of light. Radiant energy of 21,000 ergs/cm^2 · sec was applied in these experiments. Violet light (427 mμ) reversed the response in a negatively reacting population. When the same population after this transformation was illuminated with yellow light (590 mμ) the response changed to negative, and consecutive illumination with red light (685 mμ) induced positive response. The induction time at these wavelengths was around 5 min. This sequence could be repeated for several hours. When the cells at the end of such a series of changes were continuously illuminated with the yellow light, the cells remained negative for at least 14 h, and similarly red illumination produced a positively reacting population.

These action spectra determinations suggest that two light processes may be involved in induced phototactic response changes in *Platymonas*. Photosynthesis seems to be one of these. This conclusion is founded on the effect of violet and red light. In these spectral regions the action spectrum of induced positive response shows great similarity to action spectra of photosynthesis of green algae. As the effect at the

References p. 126

500-mμ light seems to be too high to fit the photosynthesis curve, it is assumed that the pigment directly involved in photic orientation is also involved in the transformation process. The negative to positive transformation is antagonized by a reaction involving a pigment which absorbs maximally around 590 mμ. This indicates that a reversible photoreaction, possibly one associated with a pigment system with similarities to that involved in photoperiodism, is directly involved in the mode of phototactic response in *Platymonas*.

REFERENCES

1 W. HAUPT, *Die Phototaxis der Algen*, In *Encyclopedia of Plant Physiology* (W. RUHLAND, ed.), Vol. XVII, Part 1, p. 318, Springer Verlag, Berlin, 1959.
2 S. W. BENDIX, *Botan. Rev.*, 26, No. 2 (1960) 145.
3 P. HALLDAL, *Physiol. Plantarum*, 11 (1) (1958) 118.
4 K. SHIBATA, A. A. BENSON AND M. CALVIN, *Biochim. Biophys. Acta*, 15 (1954) 461.
5 I. GÖSSEL, *Arch. Mikrobiol.*, 27 (1957) 288.
6 E. BÜNNING AND G. SCHNEIDERHÖHN, *Arch. Mikrobiol.*, 24 (1956) 80.
7 S. O. MAST, *J. Exptl. Zool.*, 22 (1917) 471.
8 P. HALLDAL, *Physiol. Plantarum*, 14 (1) (1961) 133.
9 G. M. CURRY AND H. E. GRUEN, *Proc. Natl. Acad. Sci. U.S.*, 45 (1959) 797.
10 M. DELBRÜCK AND W. SHROPSHIRE JR., *Plant Physiol.*, 35(2) (1960) 194.
11 P. HALLDAL, *Physiol. Plantarum*, 12(4) (1959) 742.
12 P. HALLDAL, *Physiol. Plantarum*, 13 (4) (1960) 726.

PHOTOTROPISM; THE NATURE OF THE PHOTORECEPTOR IN HIGHER AND LOWER PLANTS*,**

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In the majority of growing plants, asymmetrically incident light will cause a growth redistribution leading to light-oriented movement of the growing parts, the phenomenon of phototropism. In higher plants there is strong evidence that phototropic curvatures result from light-induced redistribution of auxins. It has long been known that phototropism is largely a response to blue wave lengths, so that we must postulate the existence of a yellow pigment which, upon absorption of light, changes the pattern of growth, most probably by interacting with the auxin system.

Two major approaches have been used in attempts to identify the photoreceptor. The first involves the disarmingly simple question: What yellow pigments are found in the phototropically sensitive parts of plants? In answer to this, various workers early found substantial concentrations of carotenoid pigments associated with the photosensitive regions of plants such as *Avena*, *Pilobolus*, and *Phycomyces*. This led directly to the postulation that a carotenoid is the photoreceptor.

Evidence has been accumulating, however, that plant parts may exhibit phototropism and yet have extremely low carotenoid content, as in coleoptiles of corn and barley albino mutants, germ tubes of a number of fungi[1], "colorless" rhizoids of certain liverworts[2], and the coremia of a white *Isaria*. Furthermore, the distribution of total carotenoids in the plant does not seem to parallel the distribution of light sensitivity as precisely as one might expect. The best case of this is seen in the *Avena* coleoptile, where Lange[3] found maximum photosensitivity in the apical 1/4 mm, while Bünning[4] found maximum free carotenoid concentration just below this. The extreme apex has a very low carotenoid content. In our laboratory Dr. Sorokin has recently confirmed this distribution of crystallizable carotenoid as shown in Fig. 1.

Meanwhile, other yellow pigments, notably flavins, have been found in photosensitive organs, although not specially concentrated there. With Galston's demonstration that riboflavin could photosensitize the *in vitro* photo-oxidation of the auxin indoleacetic acid, attention became especially focussed on the possibility that a flavin acts as photoreceptor.

The question of the occurrence of a yellow pigment in photosensitive regions is obviously oversimplified. We have no idea what concentration of pigment may be required to convey phototropic sensitivity to the plant. Even if the receptor is a carotenoid, in the best cases only a very small amount is present, and perhaps only a

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References p. 134

small fraction of this need be active. Furthermore, no simple criterion of "sensitivity" exists, especially when comparing different plant groups. Thus, many of the carotenoid-deficient plants mentioned before seem phototropically sluggish in comparison with *Avena*, but we cannot say whether this reflects normal or abnormal phototropic sensitivity. Some albino mutants reportedly show normal phototropic sensitivity, yet, for example, Asomaning's data[5] indicate that in an albino barley variety having approxi-

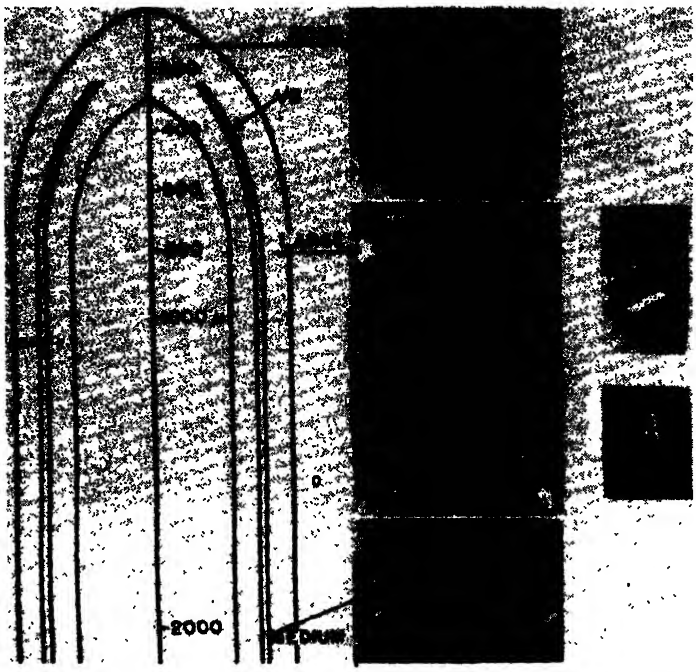

Fig. Distribution of crystallizable carotenoid in the tip of the *Avena* coleoptile. At the right: Crystals at a larger magnification, *ca.* 1500 ×.

mately 1/3 the normal amounts of carotenoid, the phototropic sensitivity is also distinctly lower. Similarly, he found that etiolated barley coleoptiles have only 1/15 as much carotenoid as etiolated oat coleoptiles, and give no curvature whatsoever in response to short stimuli which will produce large curvatures in oats. Parenthetically, no equivalent variations in the flavin content were found in these cases.

All in all, we can only comment on the peculiar localization of carotenoid ***in or near*** the photosensitive regions of many highly phototropic plants. The complexities outlined above lead us to the second major approach — the action spectrum. Its interpretation should not depend so much on pigment concentration or localization; if sufficiently detailed, it must reflect the absorption spectrum of the photoreceptor, although it may possibly be distorted by inactive absorbers.

Early studies of the wave length dependence of phototropism, mostly using null-point techniques, indicated the presence of two peaks in the blue region[6,7]. A more flexible procedure is to determine the dose-response curve at each wave length. Strangely enough, *Avena* is the only case so far where curvature has been found to be clearly graded with respect to light dosage. Our data for two wave lengths are shown in Fig. 2; they illustrate the well-known periodic behavior of *Avena*. Following low light dosages the coleoptile responds in an hour or so by developing a positive curva-

ture. Over a 10- to 100-fold range of dosages these curvatures are fairly simply related to the amount of incident light; in fact, over a portion of this range the response is nearly log-linear. These so-called "first positive" curvatures are small, rarely greater than 30°, and in the first hour are concentrated near the tip of the coleoptile. With still larger dosages, however, smaller curvatures may result; *i.e.*, the response curve passes through an optimum. Beyond this optimum the curve falls steeply, and in a

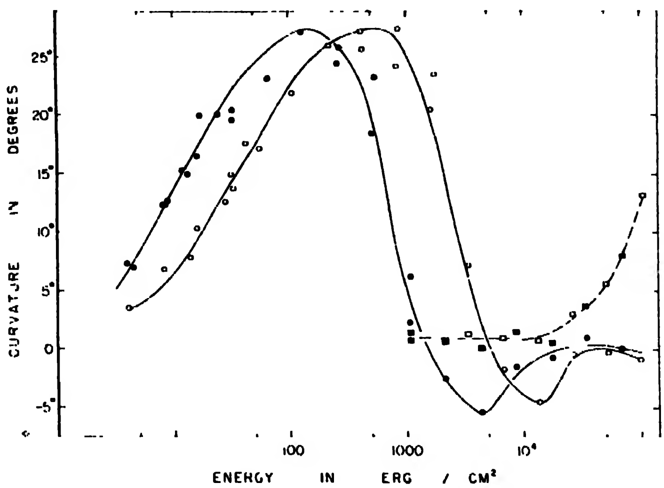

Fig. 2. Response curves of *Avena* at two wave lengths. Ordinates: curvature after 90 min; abscissa: total light dose. ● tip curvature, 436 mμ; ■ base curvature, 436 mμ; ○ tip curvature, 365 mμ; □ base curvature, 365 mμ.

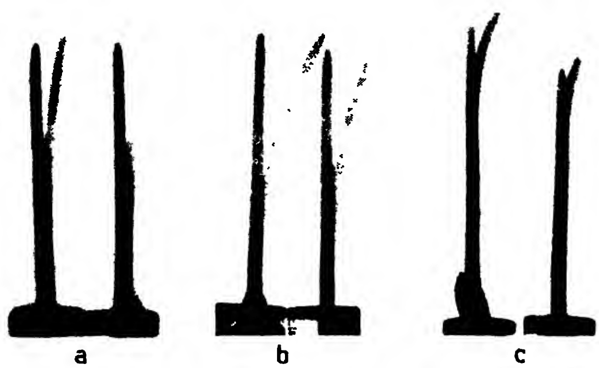

Fig. 3. Typical curvatures of *Avena* coleoptiles caused by light. a, base response caused by 10 min of medium intensity blue light (436 mμ). b, base response caused by 10 sec of ultraviolet light (254 mμ). c, tip response caused by 1 sec of blue light of the same intensity and wave length as in a. (Direction of light from the right. All shadowgraphs taken at 0 and 90 min from treatment.)

range of dosages obtained by applying very high intensities for a second or two, the response may actually be negative, bending away from the light. For prolonged stimuli, usually 4 min or longer, a "second positive" response follows, qualitatively different from the first positive: curvatures are distributed along the entire length of the coleoptile, originating in or near the mesocotyl. They may be alternatively described as "base responses". Their magnitude depends largely on the duration of

References p. 134

the exposure rather than the product of intensity and duration. Thus, even low intensity light may yield a base response if applied for longer than 4 min. Fig. 3 illustrates these types of response.

Returning to Fig. 2, we can draw the following conclusions. First, the relative effectiveness of various wave lengths can only be assayed by determining the whole response curve or by carefully selecting as a standard a given response on the rising part of the first positive range and comparing the energies required to give this response. Second, the fact that the response curves at two widely different wave lengths run parallel over most of their course argues that a single photoreceptor underlies both the first positive and negative curvature processes. The fact that both of these curvatures are quite restricted to the tips also supports the idea of a similar mechanism. On the other hand, the response curves obtained with prolonged exposures are superimposed and the response itself is qualitatively different. The superimposition seems to be a consequence of the fact that the response in this range is time-dependent rather than dose-dependent. These facts suggest that the second positive type of phototropism in *Avena* may or may not involve the same photoreceptor as the first positive, but must, in any case, involve a different mechanism.

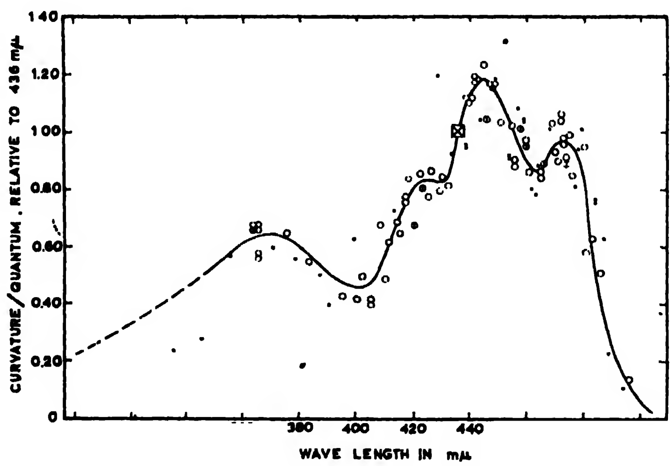

Fig. 4. Action spectrum for the tip response of *Avena* coleoptiles. ● average 1 row at λ; ○ average 4 rows at λ; ⦶ average 6 rows at λ; 8 average 8 rows at λ; etc.

We have made a detailed determination of the relative numbers of quanta required at each wave length to give a 10° first positive response in *Avena*. The results are shown in Fig. 4. The existence of two main peaks in the blue is confirmed. Separate experiments with large numbers of plants exposed to 443, 458, and 473 mμ have confirmed the statistical significance of these two peaks. A definite shoulder appears below the principal maximum and a small peak in the near ultraviolet.

This spectrum agrees in all major respects with the action spectrum found simultaneously and independently by Shropshire and Withrow[8]. They found, however, that the slope of the response curve varied for each wave length so that the action spectrum depended upon the magnitude of the standard response selected for analysis. In essence, the action spectrum for 20° curvatures has the same peaks in the blue but the peak in the near U.V. is much accentuated. On the other hand, by extrapolating

the response curves to zero curvature, they found that the action spectrum for threshold curvatures lacks the U.V. peak altogether.

Although we have found it difficult to characterize the near U.V. peak precisely, we have not been able to confirm that the slope of the response curve is indeed a function of wave length; *i.e.*, we find no significant difference in these slopes. Furthermore, since the response curves are log-linear only in a restricted range, extrapolations to an apparent threshold may be inapplicable. This matter is dealt with in detail in another session of the Congress. In any case, three peaks in the blue region of the action spectrum and a minor peak in the near ultraviolet seem now well substantiated.

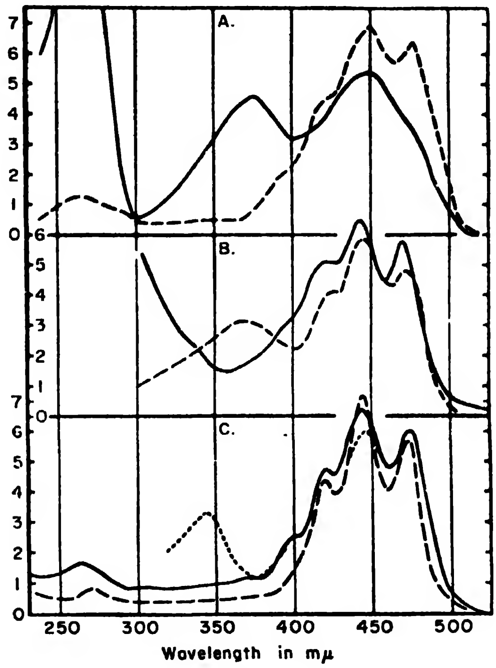

Fig. 5. Comparison of action spectrum and absorption spectra. (All ordinates are arbitrary.) A, Solid line: absorption spectrum of riboflavin. Broken line: absorption spectrum of β-carotene in hexane. B, Solid line: absorption spectrum of a direct hexane extract of 500 coleoptile tips. Broken line: action spectrum for tip response in *Avena*. C, Solid line: γ-carotene in hexane. Broken line: lutein in hexane. Dashed line: the *cis*-peak of a mono-*cis*-isomer of β-carotene.

These action spectra are strikingly similar to the absorption spectra of carotenoid pigments from the coleoptiles. If the near U.V. peak did indeed disappear in the threshold curve, which, according to Shropshire and Withrow, is the curve least likely to be distorted by masking or by selfscreening, then the similarity to carotenoid absorption would become even more pronounced. This is a critical point, because, as mentioned previously, the other most reasonable candidate for photoreceptor is a flavin. Flavins generally possess a single maximum in the blue, rather than three, and they have a pronounced peak, usually higher than the blue peak, in the near U.V. These relationships are shown in Fig. 5. Recently it has been found that, when dissolved in certain non-polar solvents, some flavins exhibit three absorption peaks in the blue[9]. These still have the near U.V. peak, but this is shifted 30 mμ toward shorter wave lengths.

References p. 134

On the other hand, *cis*-isomers of the more common all-*trans*-carotenoids frequently show peaks in the near U.V., although these, too, are usually 30 to 40 mμ below the 370 peak of the action spectrum. In sum, we can say that no known flavin in *Avena* has been found to show the three peaks in the blue shown by the action spectrum, nor is the observed U.V. peak, if real, as large as one would expect if a flavin were the photoreceptor. Carotenoids actually present in *Avena*, however, match the blue peaks strikingly, but none has yet been found possessing the maximum in the near U.V. at the right wave length. The possibility remains that this peak may reflect a special structural configuration of the pigment in the plant, which is lost upon extraction. These action spectra do not unequivocally indentify the photoreceptor.

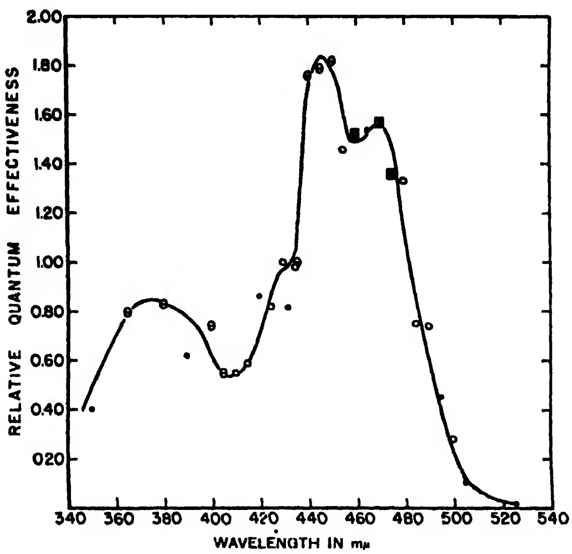

Fig. 6. Action spectrum for the phototropism of *Phycomyces* sporangiophores. ● single determination; ○ 2 3 determinations; ⊖ 4 -5 determinations; ⊠ 6 or more determinations.

Detailed action spectra have also been obtained for the sporangiophores of *Phycomyces*. Here the plant shows extremely limited first positive curvatures; we have at best obtained 7° with short exposures. The sporangiophores are phototropically indifferent to short, high intensity exposures as noted by Castle[10] in 1931. Growth accelerates temporarily after such stimuli; *i.e.*, the sporangiophores undergo a light-growth-reaction, but no curvature develops. Castle interpreted this as a light saturation effect in which both sides of the organ are presumably fully stimulated when exposures exceed certain dosages. Yet curvatures do develop when the exposures are continuous; we do not know how this is mechanistically relatea to curvatures produced by short exposures. Apparently some enduring asymmetry in growth, quite distinct from the light-growth-reaction, is established by prolonged light exposures.

This behavior necessitates the use of a null-point method for determining the action spectrum in *Phycomyces*[11,12]. In essence, we find for various wave lengths the light flux required to balance that from a standard source impinging from the opposite side. In the visible range we obtained the action spectrum shown in Fig. 6. A similar, though less detailed, spectrum has recently been obtained by Delbrück and Shropshire[13]. The action spectrum for *Phycomyces* is almost identical with that of *Avena*, including

the presence of a near U.V. peak less than half the height of the blue maximum. Thus the photoreceptors in these two morphologically and systematically different plants are similar if not identical.

It has been argued recently[14,15] that a carotenoid-like action spectrum may be a consequence of a "masking" or "internal screening" effect of large concentrations of carotenoid pigments in the presence of relatively low amounts of the actual photosensitizing pigment, usually assumed to be a flavin; thus the high phototropic sensitivity of *Avena* tips would be due to the optical filter action of the carotene present, which would produce the required gradient of light absorption across the organ. We have examined this idea in detail and calculated the effects to be expected from various model arrangements of "active" and "screening" pigments. No matter what model

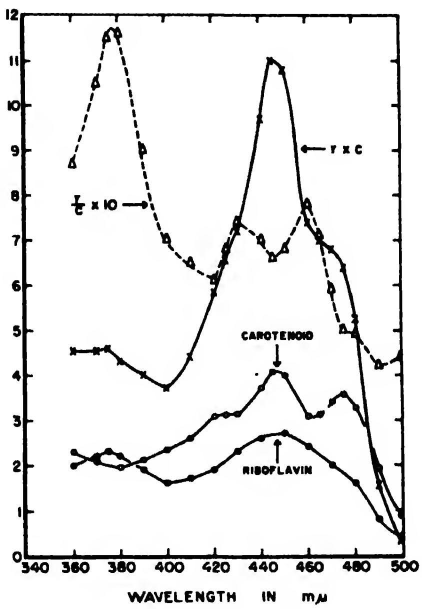

Fig. 7. Action spectra to be expected in limiting cases of masking of "active" riboflavin by "inactive" carotenoid. The absorption spectra of the two components are shown in the lower part of the figure. *r* and *c* in the upper curves refer to the absorption coefficients of the riboflavin and of the extract of total carotenoid respectively, in the lower curves. Ordinate is arbitrary.

system is chosen, two limiting cases seem to arise, the first case predicting that the action spectrum should reflect the *product* of the absorption spectra of the active and inactive pigments, and the second case the *quotient* of the two. These two functions are plotted for limiting cases of masking of "active" riboflavin by "inactive" carotenoid in Fig. 7. It is apparent that neither case leads to an action spectrum that at all resembles the one actually observed.

The masking argument also fails to explain why *Avena* and *Phycomyces* should exhibit almost identical action spectra, when they must surely possess quite different arrangements of active and inactive pigments. Bearing on this point, Delbrück and Shropshire conclude that there is no appreciable distortion of the action spectrum in the visible in *Phycomyces*, though a very strong one, presumably in the near U.V., in *Avena*. It seems more reasonable to us to ascribe the near U.V. peak to distortion of

References p. 134

carotenoid absorption by flavin masking, rather than postulating that the peaks in the blue arise from screening by carotenoids. Moreover, the reportedly high phototropic sensitivity of some carotenoid-low mutants is impossible to reconcile with the masking theory, since on this theory the sensitivity presumably depends on the gradient imposed by the masking pigment. Similarly, the previously mentioned discrepancy between localization of carotenoids and distribution of photosensitivity is also inconsistent with the masking theory. We conclude that, while the presence of an inactive pigment may increase the gradient, it can only modify the action spectrum to a limited extent and will not transform a flavin spectrum into a carotenoid spectrum, even in the extreme case.

The action spectrum for the light-growth-reaction of *Phycomyces* is practically identical to that for phototropism. Less-sensitive organisms are difficult to deal with and have not been studied in detail, but most, including *Isaria*, have shown some sensitivity to near U.V., a maximum sensitivity to blue, and a sharp cut-off between 500 and 550 mμ. Therefore, there seems to be a remarkably similar pattern for the phototropic photoreceptors of all plants. Whether the number of effective photoreceptors is indeed so limited can only be determined when more are investigated in detail.

In the ultraviolet range between 200 and 300 mμ we feel that a different photoreceptor may be implicated. In this range the responses are qualitatively different from those in the visible, consisting of base curvatures in *Avena* and strongly negative curvatures in *Phycomyces*. The action spectrum for *Avena*[16] has a peak or peaks near 290 mμ, while that for *Phycomyces*[12] is maximal near 280 mμ. It must be pointed out that the flavin photoreceptor hypothesis does not fit these curves well, since most flavins have a very large absorption peak in the 265-mμ range, well below the peaks in the action spectra and indeed near their troughs.

In conclusion, we feel that the weight of evidence derived from action spectrum studies favors the carotenoid photoreceptor hypothesis. The specific identification of the photoreceptor awaits the discovery within the plant of a demonstrably light sensitive pigment having properties accounting for the action spectrum and linked somehow to the growth system.

REFERENCES

1 G. GETTKANDT, *Wiss. Z. Martin-Luther-Univ., Halle-Wittenberg*, 3 (1954) 691.
2 M. DASSEK, *Beitr. Biol. Pflanz.*, 26 (1939) 125.
3 S. LANGE, *Jahrb. wiss. Botan.*, 67 (1927) 1.
4 E. BÜNNING, *Planta*, 26 (1937) 719; 27 (1937) 148.
5 E. J. A. ASOMANING, *Phototropic Behavior and Pigment Content of Grass Coleoptiles*, Dissertation, Yale Univ., New Haven, Conn., 1960, pp. 1–114.
6 F. BACHMANN AND F. BERGANN, *Planta*, 10 (1930) 744.
7 E. S. JOHNSTON, *Smithsonian Inst. Publs., Misc. Collections*, 92 (1934) 1
8 W. SHROPSHIRE JR. AND R. B. WITHROW, *Plant Physiol.*, 33 (1958) 360.
9 H. A. HARBURY, K. F. LA NOUE, P. A. LOACH AND R. M. AMICK, *Proc. Natl. Acad. Sci., U.S.*, 45 (1959) 1708.
10 E. S. CASTLE, *Botan. Gaz.*, 91 (1931) 206.
11 E. S. CASTLE, *J. Gen. Physiol.*, 14 (1931) 701.
12 G. M. CURRY AND H. E. GRUEN, *Proc. Natl. Acad. Sci., U.S.*, 45 (1959) 797.
13 M. DELBRÜCK AND W. SHROPSHIRE JR., *Plant Physiol.*, 35 (1960) 194.
14 J. REINERT, *Z. Botan.*, 41 (1953) 103.
15 L. BRAUNER, *Ann. Rev. Plant Physiol.*, 5 (1954) 163.
16 G. M. CURRY, K. V. THIMANN AND P. M. RAY, *Physiol. Plantarum*, 9 (1956) 429.

CYTOCHROME OXIDATION BY A SECOND PHOTOCHEMICAL SYSTEM IN THE RED ALGA *PORPHYRIDIUM CRUENTUM**

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Illumination of photosynthesizing cells causes changes in the absorption spectrum. Some of these changes were found to be due to biological catalysts, such as cytochromes. Illumination caused oxidation of one or more cytochromes in all purple bacteria studied so far.

In algae there is a decrease in absorption upon illumination around 420 mμ, which may, at least partly, be due to cytochrome oxidation, but in many algae changes occur which make it difficult to analyse the spectral changes with certainty. However, as will be seen, in the red alga *Porphyridium cruentum*, the difference spectrum clearly shows that a cytochrome of type *c* (or *f*) is oxidized upon illumination; there appears to be no appreciable change in absorption caused by other pigments. There are also indications that more than one photochemical pigment system is present. For these reasons we have used this species for the study of the kinetics, quantum requirement and action spectrum of cytochrome oxidation.

METHODS AND MATERIALS

After inoculation from agar-agar, the algae were grown in the light of white fluorescent tubes for about seven days in a liquid culture medium[1], bubbled with air enriched with 5% carbon dioxide.

The changes in absorption were measured by means of an improved split-beam difference spectrophotometer, in principle similar to that described earlier[2]. The algal suspension was transferred to a 10 × 10 mm absorption vessel, which can be illuminated from the side with filtered actinic light from a 500-W projector or from a Bausch and Lomb monochromator illuminated with a xenon arc. The transmittance at 680 mμ for a 10-m suspension of algae, corrected for scattering by dividing by the transmittance at 720 mμ, where the real absorption is negligible, was about 80%. The intensity of the actinic beam is measured by means of a silicon cell, to which the beam is deflected by means of a movable mirror. The silicon cell (Lange) was calibrated by means of a calibrated thermopile, which was placed in the position of the absorption vessel. The intensity of the actinic beam is expressed in einstein/cm^2/sec; 1 einstein = N quanta, N being the number of molecules in a gram molecule; 10^{-11} einstein is equivalent to 17.6 ergs at 680 mμ, and to 21.3 ergs at 560 mμ. The actinic

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References p. 142

beam, which is more intense than the measuring beam, serves to cause a change in absorption of the algal suspension, but does not cause a response of the one-fourth-second spectrophotometer recorder, because a filter which strongly reduces scattered actinic light is placed in front of the photomultiplier. Furthermore the detecting apparatus is only sensitive to modulated light; the measuring beam is modulated by a rotating disc, but the actinic beam is not.

RESULTS

Time course

With the spectrophotometer set at 420 mμ, actinic light of 680 mμ, which is absorbed by chlorophyll *a*, caused a deflection of the recorder showing a decrease in optical density of the *Porphyridium* suspension. The top recording of Fig. 1 shows that upon

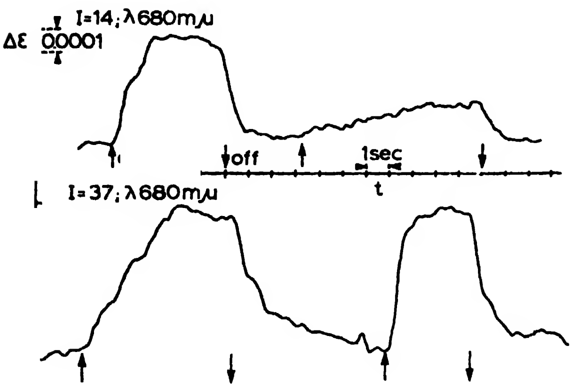

Fig. 1. The optical density at 420 mμ of a *Porphyridium* suspension, recorded as a function of time. An upward deflection represents a decrease in optical density. The actinic light of 680 mμ was given between the upward and downward arrows. The intensities, *I*, of the actinic light were $14 \cdot 10^{-11}$, and $37 \cdot 10^{-11}$ einstein/cm²/sec for top and bottom recording respectively.

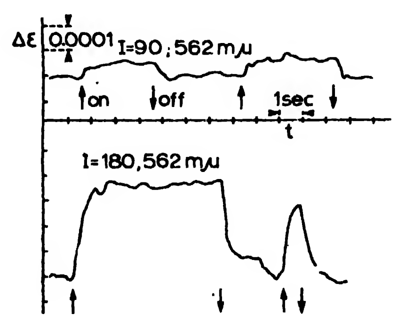

Fig. 2. Optical density changes at 420 mμ for actinic light of 562 mμ, and intensities 90 and $180 \cdot 10^{-11}$ einstein/cm²/sec. See legend of Fig. 1 for details.

onset of the weak illumination, after the algae had been in the dark for about one minute or longer, a decrease in absorption occurred within about two seconds; after darkening the absorption increased again to the original level. Upon the second illumination with the same intensity, which followed the first within a few seconds, the initial rate of decrease in absorption, and the total deflection, and also the rate of increase upon darkening, were smaller than upon the first illumination. Further illuminations, with the same intensity after a few seconds of darkness, yielded a time course, similar to the second one. After a minute of darkness, the same sequence of events could be reproduced, although not exactly. For low intensities of actinic light, it was mostly observed that the second illumination caused a decrease at a lower rate than the first, as in the recording at the top of Fig. 1. At higher intensities the opposite was the case: the recording at the bottom of Fig. 1 shows that the rate of increase is appreciably higher in the second illumination.

Quite surprisingly, the time course of the changes in absorption at 420 mμ for actinic

light of 562 mμ was quite different from that for actinic light of 680 mμ. For actinic light of 562 mμ, at an intensity of about $I = 40 \cdot 10^{-11}$ einstein/cm², no measurable deflection occurred, although, as we shall see, for light of 562 mμ, where phycoerythrin has an appreciable absorption, the rate of photosynthesis is appreciably higher than for equally intense light of 680 mμ.

A small, but relatively rapid deflection occurred at $I = 90 \cdot 10^{-11}$ einstein/cm²/sec (Fig. 2, top recording). At twice this intensity the decrease in absorption was appreciably higher (bottom part of Fig. 2).

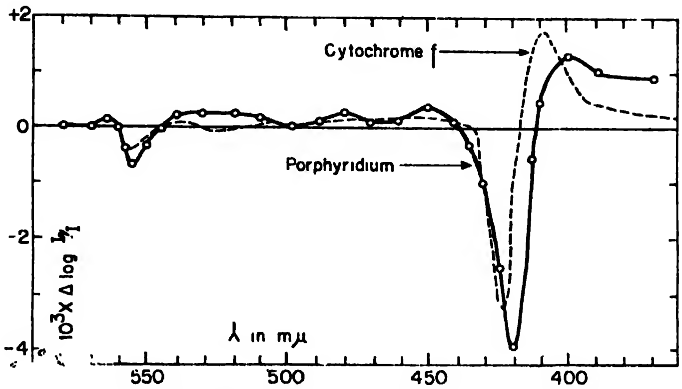

Fig. 3. Difference spectrum of *Porphyridium cruentum*, absorption spectrum in light, minus that in darkness. The difference spectrum of cytochrome *f*, oxidized minus reduced, was obtained from data of Davenport and Hill[5].

We measured the changes in absorption for a number of wavelengths of the measuring beam from 400 to 435 mμ, using actinic light of an intensity of 73 einstein/cm²/sec at 680 mμ and of 230 einstein/cm²/sec at 562 mμ. At these intensities the maximum steady state change in absorption is attained at 420 mμ. If the steady state increase in optical density, which for this relatively high intensity was found to be about the same in a few consecutive periods of illumination, is plotted as a function of the wavelength of the measuring beam, a difference spectrum is obtained, which represents the difference between the absorption spectra of the suspension in the steady state in the light and in the dark. The difference spectra in the region 400 to 435 mμ, appeared rather similar to that of Fig. 3: both spectra had a negative maximum at about 420 mμ, and showed an increase in absorption for wavelengths shorter than 410 mμ. This indicates that the maxima at 420 mμ in the spectra excited by actinic light of 680 mμ and 562 mμ are caused by the same cytochrome as that, whose difference spectrum is shown in Fig. 3. The difference spectrum shown in Fig. 3 was measured[4] for a more highly concentrated suspension under conditions different from those described in this paper.

Quantum requirement for cytochrome oxidation

Assuming that the decrease at 420 mμ was caused by one cytochrome of the *c*-type, which we shall call C 420, we estimate the number of quanta absorbed at 680 mμ which is required to oxidize one cytochrome molecule, as follows. We use the experiment of which the recording is given in the upper left part of Fig. 1. The absorptance of the suspension of 1 cm thickness at 680 mμ, corrected for scattering, was 19.5%.

References p. 142

The energy incident upon 1 cm^2/sec was $14 \cdot 10^{-11}$ einstein. Thus, the energy absorbed per ml per sec was $19.5 \cdot (1/100) \cdot 14 \cdot 10^{-11}$ einstein $= 2.7 \cdot 10^{-11}$ einstein. The initial rate of decrease of absorbancy in optical density units is read from Fig. 1 as $3.0 \cdot 10^{-4}$ per sec. The value of the change in optical density at 420 mμ upon oxidation for a *c*-type cytochrome extracted from another species of red algae[6] is roughly 70/cm/m*M*. Assuming a 30% "flattening" of the absorption spectrum of *Porphyridium*[7] and thus of the cytochrome at 420 mμ, we estimate the corresponding value of the cytochrome in intact *Porphyridium* to be 50/cm/mmole oxidized. If 1 mole of cytochrome C 420 per ml instead of 1 mmole per liter were oxidized, there would be a change in optical density units for a layer of 1 cm thickness of $50 \cdot 10^3 \cdot 10^3 = 5 \cdot 10^7$. A rate of $3.0 \cdot 10^{-4}$ optical density units per second then corresponds to $3.0 \cdot 10^{-4}/(5 \cdot 10^7) = 6.0 \cdot 10^{-12}$ moles of cytochrome oxidized per ml per sec. Since this oxidation is brought about by $2.7 \cdot 10^{-11}$ einstein, it follows that during the oxidation of one cytochrome molecule, $2.7 \cdot 10^{-11}/(6 \cdot 10^{-12}) = 4.5$ quanta are absorbed. The same number is found from the recording represented in the lower right hand part of Fig. 1 : both the intensity and the initial rate are approximately 2.7 times as great as in the experiment discussed above. Because of the uncertainty in the rate of the change in optical density and because of inhomogeneity of the actinic beam, the value 4.5 for the quantum requirement is to be considered an approximation.

Assuming the flattening of the chlorophyll absorption peak at 680 mμ to be 25%, we calculate the ratio of chlorophyll *a* molecules to that of cytochrome C 420 molecules to be about 130.

Action spectrum of cytochrome oxidation

Since the kinetics of the change in absorption at 420 mμ with actinic light of 562 mμ appear to be different from those with light of 680 mμ, an action spectrum for this change can be derived in several ways.

At 680 mμ, a number of recordings, such as those given in Fig. 1, were made for several light intensities. The time course after the second illumination was used: in Fig. 1 this is the part of the recordings at the right hand side. The deflection from darkness to steady state in the light proved to be an increasing function of light intensity. This was also true for other actinic wavelengths. A graph was made, representing the deflection at 420 mμ as a function of the actinic intensity of 680 mμ. Using the activity for cytochrome oxidation with light of 680 mμ as a reference, the activities for other wavelengths were determined as follows. After a few trials, an actinic intensity $I(\lambda)$ was selected so that the deflection was roughly half of the maximum deflection. Then from the graph for 680 mμ, the intensity $I(680)$ was read, which gave the same deflections as $I(\lambda)$. The activity $Q(\lambda)$, was defined as $Q(\lambda) = 10 \cdot I(680)/I(\lambda)$, the activity at 680 mμ being arbitrarily put equal to 10. In Fig. 4, Q is plotted as a function of λ (black points). The activity is high in the region of the chlorophyll *a* maximum around 680 mμ, but low in the region between 650 and 550 mμ, where the phycobilins show an appreciable absorption.

In an analogous way the action spectrum was determined, using the initial rate after the second illumination as a measure of the activity. This action spectrum is given in Fig. 4 by open squares.

In order to avoid a substantial weakening of the actinic light, a suspension of low absorbance was used; the absorbance corrected for scattering was 18% at 680 mμ

and 27% at 560 mμ. Because of the smallness and rapidity ot the deflections and the limited reproducibility of the time course, the precision of the action spectra (especially that of the open squares) is low.

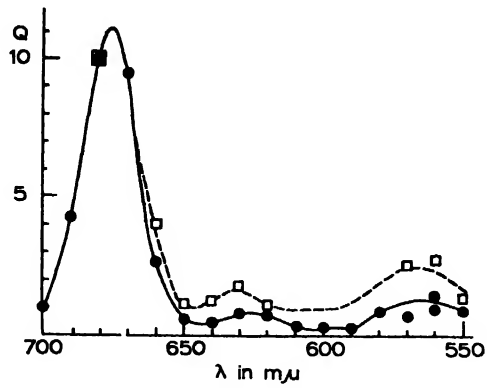

Fig. 4. *Porphyridium cruentum*. Action spectrum of cytochrome oxidation. Reciprocal values of the relative intensities of the actinic light needed to bring about a certain deflection at 420 mμ (black points), or a certain initial rate of change in absorption at 420 mμ (open squares), are plotted as a function of the wavelength of the actinic light.

DISCUSSION

Fig. 5 shows that the absorption of *Porphyridium* at 680 mμ is mainly caused by chlorophyll *a* : the absorption of this pigment is given by line segments with legend chl. At 630 mμ the absorption is mainly due to chlorophyll *a* and phycocyanin, pcy., and at 560 mμ mainly to phycoerythrin, per. The action spectrum of photosynthesis, that is of oxygen evolution, shows that light absorbed by phycocyanin and phycoerythrin is more active in photosynthesis than light absorbed by chlorophyll *a* at 680 mμ. On the contrary, the action spectrum for cytochrome oxidation (Fig. 4) suggests that light absorbed by chlorophyll *a* is more active in cytochrome C 420 oxidation than light absorbed by phycocyanin or phycoerythrin. The simplest but not complete explanation is that two photochemical systems are present. One of these, let us call it system 2, contains the main part of the phycobilins and part of the chlorophyll *a*, say chlorophyll a_2, and is mainly responsible for oxygen evolution. System 1 contains a remaining part of chlorophyll *a*, chlorophyll a_1, and a smaller part of the phycobilins and is mainly responsible for cytochrome C 420 oxidation.

The action spectrum of chlorophyll fluorescence (Fig. 5) shows that quanta absorbed by the phycobilins are more efficient in exciting chlorophyll *a* fluorescence than quanta absorbed by chlorophyll *a* itself. From this it can be concluded (*cf.*[3]) that light energy absorbed by the phycobilins must be transferred to a part of chlorophyll *a*, which is fluorescent, and further that part of the chlorophyll *a* is not, or only weakly, fluorescent, and receives an appreciable smaller amount of light energy from the phycobilins. Fig. 5 also shows that photosynthesis and chlorophyll *a* fluorescence are substantially proportional; a possible deviation from the proportionality will be discussed below. From this it was concluded[3] that probably the fluorescent type of chlorophyll *a*, to which the phycobilins transfer excitation energy, is active in photosynthesis, but not

References p. 142

the non- or weakly fluorescent type. The fluorescent type of chlorophyll thus is chlorophyll a_2, and the absorption spectrum of system 2 is identical to that of chlorophyll a fluorescence. Let us tentatively assume that the non-fluorescent type of chlorophyll is identical with the chlorophyll a_1, which oxidizes C 420.

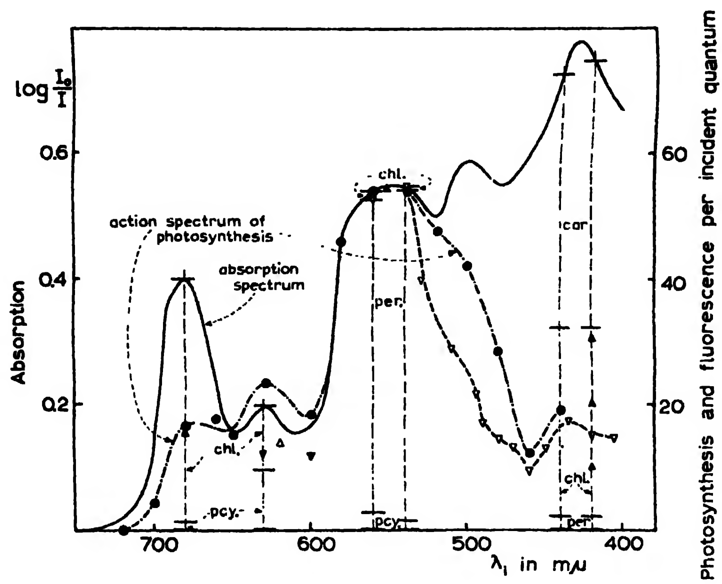

Fig. 5. Absorption spectrum, and action spectra of photosynthesis and chlorophyll *a* fluorescence (*cf.* ref.[3]). Points marked by downward-pointing open triangles are from an article by French and Young[8].

Clear experimental evidence indicating the presence of two photochemical systems in photosynthesis, which are not necessarily identical with the above hypothesized systems 2 and 1, was obtained by Emerson and coworkers[9]. Emerson observed that the relatively low photosynthetic efficiency of light absorbed by the green alga *Chlorella* at 700 mμ could be enhanced by simultaneously supplying light absorbed at shorter wavelengths. He suggested[9] that the maximum yield of photosynthesis requires not only excitation of chlorophyll *a*, but also excitation of some other pigment having its lowest excitated state of an energy level higher than that of the lowest excited state of chlorophyll *a*. In accordance with this suggestion, Myers and French[10] found that the action spectrum for the enhancement showed pronounced maxima at about 650 and 475 mμ, probably due to chlorophyll *b*. In addition, the action spectrum also showed a hump at 675 mμ, possibly due to a type of chlorophyll *a*. Brody and Emerson[11] found that the quantum yield of photosynthesis of *Porphyridium* depended upon the culture conditions, and was at 630 mμ at least as high as that at shorter wavelengths. This is in accordance with the action spectrum of photosynthesis in Fig. 5. Previously, the difference between the action spectra of the photosynthesis and of fluorescence was not given too much weight, since the action spectrum of photosynthesis which was determined with the oxygen polarograph, is relatively too low in regions of low absorption compared to regions of high absorption[3]: weakly absorbed light penetrates more deeply into the alga layer which covers the platinum

electrode than strongly absorbed light. Brody's experiments thus indicate that a downward correction of the point of the action spectrum of photosynthesis at 630 mμ may make it coincide with the absorption spectrum, but not with the triangle below the absorption spectrum. Thus there is no exact parallelism between photosynthesis and fluorescence in these algae.

Further it follows that the photosynthetic efficiency of quanta absorbed by chlorophyll *a* at 680 mμ would be less than half of that of quanta absorbed by the same chlorophyll molecules at 630 mμ (see Fig. 5). It was further observed that shorter wavelengths increase the yield from long wavelengths, just as they do in *Chlorella*. This effect has been called the "Emerson effect"[10].

Let us now, by making an additional assumption, attempt also to explain the "Emerson effect" by the above formulated working hypothesis, which was devised to explain the action spectrum of C 420 oxidation, that of photosynthesis and that of chlorophyll *a* fluorescence.

As we have seen, light absorption by system 1 alone causes cytochrome C 420 to become oxidized, but fails to bring about photosynthesis (that is in this context carbon dioxide reduction and oxygen production) with high quantum efficiency. We make the additional assumption that, if certain intermediates are generated by system 1, system 2 supplied with these intermediates will photosynthesize with higher efficiency or *vice versa*. In spectral regions, where absorption is mainly caused by system 1, the quantum efficiency of photosynthesis will drop, because system 2 is running at a low rate. This occurs around 680 mμ in *Porphyridium*, where the absorption by chlorophyll a_2 is insufficient compared with that of chlorophyll a_1. Additional illumination with wavelengths absorbed mainly by the phycobilins, that is by system 2, will then enhance photosynthetic efficiency.

The number of quanta of 680 mμ which is required to oxidize one cytochrome C 420 molecule was found to be about 4.5. Since only the quanta absorbed by chlorophyll a_1 are postulated to be active, and a_1 appears to contribute roughly half of the absorption at 680 mμ, the number of quanta required for the oxidation of one C 420 molecule, absorbed by a_1, may be of the order of two, which indicates an important physiological function for C 420. Also the relatively high molar ratio of C 420 to a_1, which is of the order of 1/70, is significant. The change in time course of C 420 oxidation, when the wavelength of actinic light is changed from 680 to 562 mμ, is in accordance with the postulated interaction of system 2 with system 1.

Analogous photochemical systems may be present in other photosynthesizing cells. In *Chlorella*, the quantum efficiency for photosynthesis is low at 700 mμ, and can be enhanced by light absorbed at shorter wavelengths[9]. As indicated by the statement of Myers and French[10] that the 695 mμ form of chlorophyll *a* is probably the chief absorber at 700 mμ, this form may be analogous to a_1. Chlorophyll a_2 in *Chlorella* would then have an absorption maximum at a somewhat shorter wavelength.

The above hypothesis is somewhat different from that of Emerson[10]. If we may express his ideas explicitly in the language of photochemical systems, Emerson's suggestions are as follows. System 2 contains a part of the so-called accessory pigments, but not chlorophyll *a*. System 1 contains chlorophyll *a* and (to explain the results of fluorescence studies) the other part of the accessory pigments. If this is true, then the action spectrum of cytochrome oxidation (Fig. 3) would necessitate the postulation of a third photochemical system in *Porphyridium*. Furthermore the systems 2 for various

References p. 142

groups of algae would operate with different photochemically active pigments, such as chlorophyll *b* in green algae, phycobilin in red algae and blue algae and chlorophyll *c* in diatoms. Also the relatively low fluorescence yield of chlorophyll *a* in red (8.3) (*cf.* Fig. 5) and in blue algae in spectral regions where chlorophyll *a* shows strong absorption, would require an additional explanation.

Identification of different photochemical systems by determination of precise action spectra and time courses under various experimental conditions, also for reactions of other substances than cytochrome C 420, in various photosynthesizing organisms seems possible. Because of the spatial separation of the photochemical systems, suggested by the preferential transfer of energy from phycobilins to chlorophyll a_2, isolation by extraction might be feasible with the absorption changes as a guide.

REFERENCES

1 M. Brody and R. Emerson, *Am. J. Botany*, 46 (1959) 433.
2 L. N. M. Duysens, in H. Gaffron (Editor), *Research in Photosynthesis*, Interscience, New York, 1957, pp. 163–174.
3 L. N. M. Duysens, *Transfer of Excitation Energy in Photosynthesis*, Thesis, University of Utrecht, 1952.
4 L. N. M. Duysens, *Science*, 121 (1955) 210.
5 H. E. Davenport and R. Hill, *Proc. Roy. Soc., (London)*, B 139 (1952) 327.
6 S. Katoh, *Plant and Cell Physiol.*, 1 (1960) 91.
7 L. N. M. Duysens, *Biochim. Biophys. Acta*, 19 (1956) 1.
8 C. S. French and V. K. Young, *J. Gen. Physiol.*, 35 (1952) 873.
9 R. Emerson, *Ann. Rev. Plant Physiol.*, 9 (1958) 1.
10 J. Myers and C. S. French, *Carnegie Inst. Wash., Yearbook*, 58 (1959) 318.
11 M. Brody and R. Emerson, *J. Gen. Physiol.*, 43 (1959) 251.

SPECTRAL SENSITIVITY MEASUREMENTS OF THE EYES OF INSECTS*,**

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The spectrum visible to many insects extends from the red through the near ultraviolet. Behavioral experiments with trained honeybees (*Apis mellifera*), performed by several investigators, notably Daumer[1], show that the honeybee discriminates colors. A simple interpretation of these data is that the honeybee possesses three systems of receptors, maximally sensitive in the green (or yellow), blue-violet, and near ultraviolet regions of the spectrum.

Action spectra are available from two sources: observations of phototaxis and other behavioral responses of worker bees[1,2], and measurements of retinal action potentials (electroretinograms)[3,4]. Both lines of evidence indicate receptors maximally sensitive in the green and near ultraviolet. Further, electrophysiological measurements of the spectral sensitivities of drone bees indicate also a blue-violet receptor.

The color vision of insects has recently been reviewed extensively elsewhere[5,6].

REFERENCES

1 K. DAUMER, *Z. vergleich. Physiol.*, 38 (1956) 413.
2 L. M. BERTHOLF, *J. Agr. Research*, 43 (1931) 703.
3 T. H. GOLDSMITH AND P. R. RUCK, *J. Gen. Physiol.*, 41 (1957-58) 1171
4 T. H. GOLDSMITH, *J. Gen. Physiol.*, 43 (1959-60) 775.
5 T. H. GOLDSMITH, in W. A. ROSENBLITH (ed.), *Sensory Communications*, John Wiley, New York, 1961.
6 T. H. GOLDSMITH, in W. D. McELROY AND B. GLASS (eds.), *Light and Life*, a Symposium of the McCollum-Pratt Institute, The Johns Hopkins University Press, Baltimore, Md. 1961.

* Invited paper.

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ACTION SPECTRA IN HUMAN SKIN*

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Working out action spectra on human skin presents great difficulties, for human skin seems to be relatively unresponsive material compared with, let us say, paramecia.

Because of this difficulty there is only one well-attested action spectrum on the effect of light on the human skin, that of the normal sunburn or erythemal reaction resulting from U.V., where it is possible to use the emission of the mercury vapour or hydrogen arc as the light source for a monochromator. Various workers have studied action spectra for this response, and their results agree fairly well (Hausser and Vahle[1]; Hausser[2]; Coblentz, Stair and Hogue[3]; Hamperl, Henschke and Schulze[4]). There is one maximum at about 300 mμ, just within the range of terrestrial sunlight, where the threshold dose is of the order of 10^5 ergs/cm^2. Another maximum is at about 250 mμ. The erythemal effects of U.V. above 320 mμ are relatively feeble and not so well known, and below 230 mμ nothing is known until the X-ray region is reached.

Another effect of U.V. is pigmentation, which may be either immediate or delayed. Firstly, *immediate* or so-called direct pigmentation, also known as pigment darkening. This is not preceded by erythema and is apparent immediately after irradiation. It persists for only a few hours or days in our experience, though other workers have noted its persistence for weeks or months (Henschke and Schulze[5]). The action spectrum is said to lie between 300 and 450 mμ, and our experience of this reaction and its action spectrum is more or less in accordance with this. It is reported that the peak of the action spectrum is at about 340 mμ, where a dose of the order of 10^8 ergs/cm^2 is required. This phenomenon is difficult to study and would merit further investigation, especially from the point of view of its action spectrum as obtained with a monochromator, as no studies have been reported on this aspect of it for over 20 years. Secondly, the much more familiar *delayed* type of pigmentation. This shows up a day or so after the sunburn response, is apparently a post-erythemal effect, and persists for many weeks or months. It is due to the formation of new melanin, and, although much is known about the biochemistry of melanogenesis, its action spectrum is one thing that has not been properly defined (Fitzpatrick and Szàbo[6]; Lerner and Case[7]). Possibly it lies between 280 and 320 mμ, that is, it resembles the action spectrum of natural sunburn.

Another response of the skin to U.V. is thickening of the horny layer of the epidermis (Miescher[8]). Again, its action spectrum is not known, but there is reason to suppose that it is similar to that for sunburn. Other effects of U.V. on human skin, such as the formation of Vitamin D, will not be dealt with here.

* Invited paper.

So much for the known normal effects of light on human skin, and the few facts of their action spectra. As Finsen was a dermatologist, I want to take this opportunity of mentioning data on abnormal action spectra, in so-called photosensitization of human skin. This is a new field in medicine and dermatology, we have much to learn in it, and, in view of this, our findings must be regarded as preliminary. I will be quoting some results obtained by Drs. Alexander, Buck, Johnson, Porter, and myself with a large quartz monochromator with a 2-kW high-pressure xenon arc as light source. Dispersion is obtained with a water prism. The width of the spectrum at the plane of the exit slit is 6 cm between 240 and 400 mμ. At 300 mμ it is possible to use wavebands of 2 3 mμ width for irradiation experiments on skin, and exposures of less than a second may be sufficient to produce a sunburn response (Magnus, Porter, McCree, Moreland and Wright[9]).

What use are action spectra in medicine? Very little at the moment. They provide for dermatologists a rational basis for the choice of external applications to prevent U.V. penetrating the skin, but very little else. We hope, however, that they will help us understand the mechanism underlying abnormal reactions of skin to light and that they might help us in diagnosis.

To illustrate the use of action spectra in this field, I will be describing some experiments with furocoumarins and pitch, and some results of tests on patients with *porphyria* and with *solar urticaria*.

Furocoumarins

These compounds, present in many plants, are well known to increase the erythemal sensitivity of human skin to long-wave U.V. This was first shown by Kuske[10].

The action spectrum of one of these furocoumarins, 8-methoxypsoralen, has been worked out by us. Spectral reactivity extends from 320 to 370 mμ with a maximum

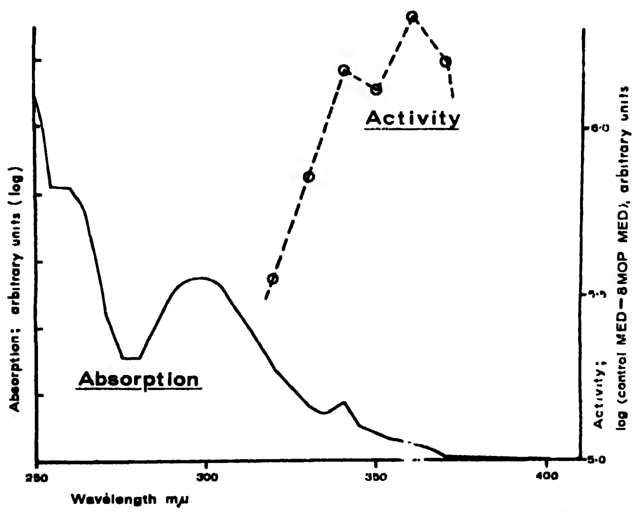

Fig. 1. The absorption and action spectra of 8-methoxypsoralen (8-MOP) compared. Left hand curve (absorption spectrum) from 8-MOP in chloroform solution. Right hand curve (action spectrum) shows erythemal activity of 1% 8-MOP (dissolved in chloroform) on human skin; average result of 3 normal subjects studied from 250 to 450 mμ. No significant difference between normal and 8-MOP treated skin from 250 to 310 mμ and from 380 to 390 mμ. All tests negative from 400 to 450 mμ whether 8-MOP treated or not.

References p. 148

perhaps at 360 mμ (Buck, Magnus and Porter[19]). This is interesting, as the action and absorption spectra of this furocoumarin are quite different (Fig. 1). However, our findings fit in with the prediction of Pathak and Fellman[11] who suggested that, on the basis of the wavelengths *activating* fluorescence, 8-methosypsoralen should be biologically active at 360 mμ. If these findings are confirmed, they may make us use the Grotthus–Draper principle, the so-called first law of photochemistry, with more caution. Apparently, as far as human skin is concerned, the furocoumarin molecule is more biologically active when in a state of fluorescence rather than when in a state of absorbing light maximally.

Is the mechanism underlying this photosensitising effect photodynamic, in that oxygen is concerned in the early stages of the reaction? This is not clear, but so far we have failed to prevent it by irradiating forearm skin rendered anoxic by stopping the blood supply with a sphygmomanometer cuff.

The photosensitising effects of pitch

This has been known for a long time as an industrial hazard (Lewin[12]), and has been investigated by Burckhardt[13] and Foerster and Schwartz[14], but no detailed action spectra have been published. Clearly, the spectral reactivity will vary according to the origin of the pitch, upon which will depend the content of photosensitizing substances.

We first became interested in this by being consulted about an industrial process in which the workers were getting troubled from burning and redness of the skin after exposure to pitch fumes and sunlight. We were able to reproduce these symptoms in

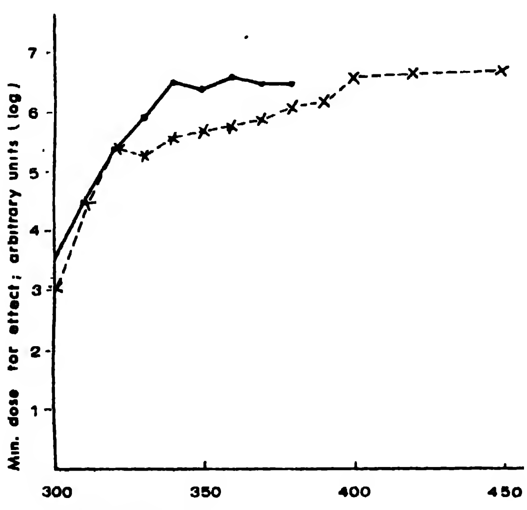

Fig. 2. Example of results on one normal subject of irradiation tests on two skin areas of the back, one repeatedly painted with pitch and cleaned with ethanol immediately prior to irradiation, the other cleaned with ethanol only. Photosensitization produced by pitch principally in long U.V. and extending well into the visible region. Tests from 500 to 700 mμ negative whether pitch-treated or not. Minimal erythemal doses (MED) are plotted for erythemal reactions that persisted for at least 12 h. In the results of the test subject illustrated, there was increased erythemal reactivity from pitch at 300 mμ, but this was not found in other normal subjects, where, in addition, tests were extended down to 250 mμ, with negative results. x— - - —x MED with pitch; ●——● normal MED.

miniature, both the burning sensation and erythema, by irradiating with the monochromator skin that had been painted with pitch. The erythemal reactions were often followed by delayed pigmentation about 2 days after irradiation. In some instances the skin responses were of an immediate urticarial type, as well as being later erythemal, a type of response noted by Foerster and Schwartz[14]. We found the action spectrum generally lay in the longer wave U.V. and adjoining visible spectrum. These spectral regions have little or no erythemal effect on normal skin unless relatively large doses of radiation are given (Fig. 2).

We have also been able to produce abnormal responses by painting the skin with anthracene, acridine and various therapeutic preparations containing tar products. The reactions are generally erythemal, sometimes urticarial, and always associated with burning. In the case of anthracene, urticarial responses, associated with burning, can be particularly readily produced by irradiation between 340 and 380 mμ. At 360 mμ a dose of the order of 10^6 ergs/cm^2 may be sufficient to produce an immediate wheal, whereas as in untreated control skin a dose of about a hundred times higher is needed to produce merely erythema. Obviously anthracene and acridine are not the sole sensitisers in pitch as their spectrally active range is too small, for with pitch we can produce photosensitization with visible light.

These abnormal reactions are presumably photodynamic for they can usually be completely prevented by occluding with a sphygmomanometer cuff the blood-flow to the skin during irradiation.

Next, *porphyria*, a disease in which, in many instances, skin symptoms are provoked by light. Typical skin changes, apparently precipitated by light, are erythema and bullae followed by atrophy. Endogenous photosensitisers act in this disease. The responsible substances are possibly uroporphyrin or coproporphyrin, both of which may be present to excess in the tissues of a patient. These photosensitising substances absorb light maximally at about 400 mμ, the region known as the Soret band, and also in various parts of the visible spectrum. In two patients with this disease we have found with the monochromator maximal skin sensitivity to be at 400 mμ, but sensitivity is also present in the visible spectrum (Magnus, Porter and Rimington[15]). Similar findings were reported independently by Wiskemann and Wulf[16] in a larger number of patients using a xenon arc and filter combinations for irradiation tests.

Lastly, *solar urticaria*. It is easy with a monochromator to produce action spectra on a patient with this disorder, but almost invariably it is impossible to interpret the results from the point of view of identifying the photosensitizing substance. In the First International Congress of Photobiology, Dr. Harry F. Blum (Blum[17]) proposed the classing of these patients into 2 groups, depending on whether they reacted to U.V. *or* to violet-blue light. I now want to propose adding two more groups to his classification, *firstly*, a group of patients in which *both* U.V. *and* visible light produce *urticaria*; *secondly* a type of *urticaria* produced predominantly at 400 mμ.

I know of three cases where *urticaria* has been provoked by U.V. *and* visible light, and there are several other cases in the literature, such as the one reported by Wiskemann and Wulf[18]. As I have said we cannot as yet make any firm interpretation of the action spectra obtained in these patients who are sensitive over these somewhat extended wavelength ranges. This does not apply to the last type of *solar urticaria* that I mentioned, that produced by 400 mμ, but I only know of one such patient in this category. Obviously *porphyria* is to be suspected on the basis of our previous expe-

References p. 148

rience. And in this instance subsequent biochemical investigations by Professor Rimington and Dr. Tickner proved this to be so. The interesting thing was that the cause of this patient's disease, previously quite unsuspected, was first diagnosed on the action spectrum. As far as I know, this is the only case of *porphyria* in which the diagnosis has been reached in this way, and here for the first time the action spectrum was the essential piece of evidence. In this patient, the concentration of porphyrins in his red cells was about thirty times above normal, and so it is interesting to speculate as to whether this man's skin symptoms were provoked by a direct action of light on the red cells in the skin capillaries.

Recognising the photosensitizing substance is encouraging, if only in one patient. It is hoped that the significance of the action spectra obtained in these and *other* ailments due to light may be grasped one day, and that in the future, they will play an important part in dermatological diagnosis.

ACKNOWLEDGEMENT

The author wishes to acknowledge his thanks to the Editor of the *British Journal of Dermatology* for Fig. 1.

REFERENCES

[1] K. W. Hausser and W. Vahle, *Strahlentherapie*, 13 (1922) 41.
[2] K. W. Hausser, *Strahlentherapie*, 28 (1928) 25.
[3] W. W. Coblentz, R. Stair and J. M. Hogue, *J. Research Natl. Bur. Standards*, 8 (1932) 541.
[4] H. Hamperl, U. Henschke and R. Schulze, *Naturwissenschaften*, 27 (1939) 486.
[5] U. Henschke and R. Schulze, *Strahlentherapie*, 64 (1939) 14.
[6] T. B. Fitzpatrick and G. Szabo, *J. Invest. Dermatol.*, 32 (1959) 197.
[7] A. B. Lerner and J. D. Case, *J. Invest. Dermatol.*, 32 (1959) 211.
[8] G. Miescher, *Strahlentherapie*, 35 (1930) 403.
[9] I. A. Magnus, A. D. Porter, K. J. McCree, J. D. Moreland and W. D. Wright, *Brit. J. Dermatol.*, 71 (1959) 261.
[10] H. Kuske, *Dermatologica*, 82 (1940) 273.
[11] M. A. Pathak and J. H. Fellman, *Nature*, 185 (1960) 382.
[12] L. Lewin, *Münch. med. Wochschr.*, 60 (1913) 1529.
[13] W. Burckhardt, *Schweiz. med. Wochschr.*, 1 (1939) 83.
[14] H. R. Foerster and L. Schwartz, *Arch. Dermatol. and Syphilol*, 39 (1939) 55.
[15] I. A. Magnus, A. D. Porter and C. Rimington, *Lancet*, (1959I) 912.
[16] A. Wiskemann and K. Wulf, *Arch. klin. u. exptl. Dermatol.*, 209 (1959) 454.
[17] H. F. Blum, *Proceedings of the First International Congress of Photobiology*, 1956, p. 359.
[18] A. Wiskemann and K. Wulf, *Arch. klin. u. exptl. Dermatol.*, 203 (1956) 394.
[19] H. W. Buck, I. A. Magnus and A. D. Porter, *Brit. J. Dermatol.* 72 (1960) 249.

EFFECT OF VARIOUS RAYS OF THE VISUAL REGION OF THE SPECTRUM ON THE ABSORPTION OF OXYGEN BY GREEN AND NON-GREEN PLANT LEAVES

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Absorption of oxygen in the light occurs in green as well as in other plants[1-4]. Short wave radiation activates this process much more strongly than long wave radiation or the dark. No detailed study of the influence of the intensity of the light has been performed. The data on the effect of the spectral composition and intensity of light on absorption of oxygen in green plants are sparse and frequently inconsistent.

In connection with a study of the role of the spectral composition of light in changing the redox conditions in plants[5-7] we have determined the rate of oxygen absorp-

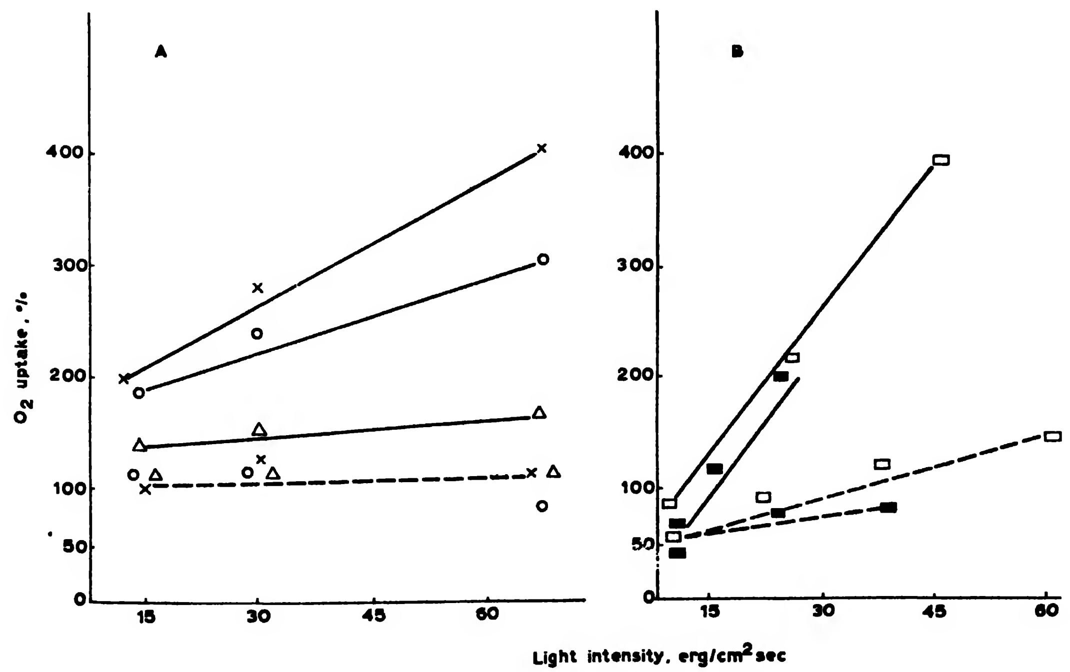

Fig. 1. Effect of light of different intensity and spectral composition on the absorption of oxygen by nonchlorophyllous and green leaves. A. Non-chlorophyllous leaves, × *Zea* mays, ○ *Aspidistra elat*, △ *Codiaeum pictum*. B. Green leaves, □ *Nicotiana tab.*, ■ *Phaseolus vulg.* Light intensity (erg/cm^2 · sec) ——— 400–580 mμ, - - - - 580–700 mμ.

References p. 152

tion by green and other leaves under illumination of various intensity and quality.

The test plants were etiolated maize shoots, nonchlorophyllous leaves of variegated varieties of Aspidistra elatior and Codiaeum pictum (which are distinguished by their containing carotinoids) and also green tobacco and bean leaves.

Absorption of oxygen was determined manometrically at a temperature of 25°. Atmospheric CO_2 and also that evolved by the leaf as a result of respiration were absorbed in 5% KOH. A part of the leaf was placed in the vessel above the hydroxide. Photosynthesis in the green leaves was thus stopped. The leaves were illuminated overhead by light of the short wave (400–580 mμ) and long wave (580–700 mμ) regions of the spectrum. The rate of oxygen absorption was first measured in the dark (control) and then in light of various intensity and quality. The results of the measurements are presented in Fig. 1. Short wave radiation was found to activate oxygen absorption in nonchlorophyllous leaves (Fig. 1A). With increase of intensity the degree of activation also increased. On the other hand, long wave radiation of arbitrary intensity had no effect on oxygen absorption which remained practically the same as that observed in the dark. The magnitude of the effect in light depended on the thickness of the leaf and was independent of the carotinoid content. Thus, short wave radiation activated absorption of oxygen to a smaller extent in the thick yellow leaves of *Codiaeum* than in the thin yellow leaves of maize. The white thick-skinned leaves of *Aspidistra* occupied an intermediate position.

A distinctive feature of oxygen absorption in green leaves (Fig. 1B) is the sensitivity of the process to long wave as well as to short wave radiation. However, activation induced by short waves is much more significant.

Thus a common feature of green and other leaves was that short wave rays activated oxygen absorption and the activation increased with growth in the intensity of the light. Differences between the two types of leaves become apparent when they were illuminated with long wave light. In this case absorption of O_2 in nonchlorophyllous leaves was the same as that in the dark. The qualitative difference between the action of the two spectral regions vanished in the case of green leaves; both regions were active but differed in that short waves were more efficient.

Since oxygen absorption is activated by short wave light in nonchlorophyllous leaves it seems natural to assume that some pigments which absorb between 400 and 580 mμ are involved. From Fig. 1A it can be seen that carotinoids do not appear to participate. On the other hand some data has been obtained which indicates that in non-green leaves, absorption of oxygen in the light involves, in particular, cytochrome

TABLE 1

Plant	*Type of experiment*	*Absorption of O_2 by 10 cm^2 of leaf area per h, μl.*		*Activation of O_2 absorption in light, %*	*Inhibition of respiration, %*	
		dark	*light 400–580 mμ*		*dark*	*light*
Zea mays, etiolated leaves	control	13.1	28.8	220		
	CO	7.0	25.8		47	11
Codiaeum leaves	control	11.5	25.8	224		
	CO	5.8	22.3		50	14
Aspidistra leaves	control	13.7	22.2	162		
	CO	7.6	16.1		45	28

oxidase. Our experiments on inhibition of the activity of cytochrome oxidase with carbon monoxide confirm this viewpoint (Table I).

As can be seen from the table in the dark CO inhibited respiration by 50%. Irradiation with short wave light removed most of the inhibiting effect as a result of destruction of the CO-cytochrome oxidase complex and restoration of the activity of cytochrome oxidase. These experiments indicate the existence of an active cytochrome oxidase in our test plants.

Experiments with sodium azide yield concurring results. As is well known sodium azide inhibits the activity of oxidases containing heavy metals. When this poison was infiltrated into the leaves in the dark (Table IIA) the absorption of oxygen was found to decrease as a result of inactivation of cytochrome oxidase and polyphenol oxidase.

TABLE II

Conditions	*Absorption of oxygen, % control*		
		Light	
		400–580 mμ	*580–700 mμ*
		A. Nonchlorophyllous leaves	
Control	100	100	100
Sodium azide, *0.002 M*	65	46	67
		B. Green leaves	
Control	100	100	100
Sodium azide, 0.007 *M*	43	96	100

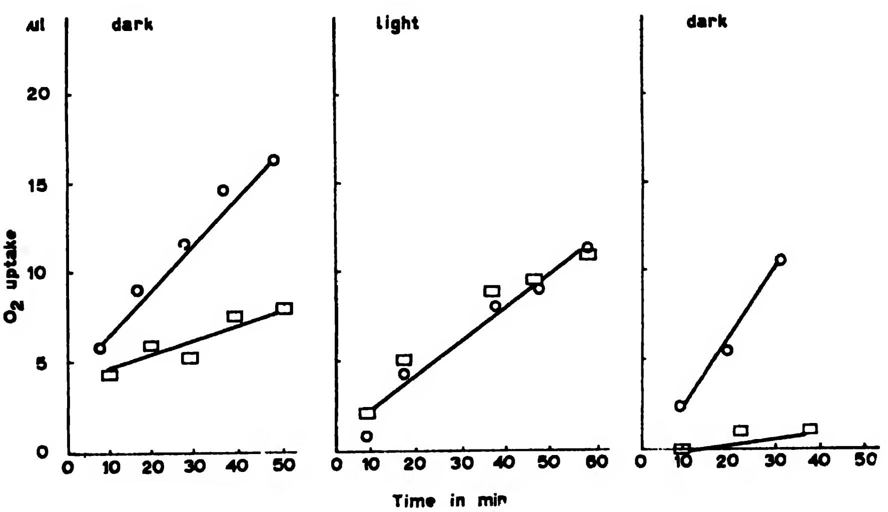

Fig. 2. Effect of sodium azide on the absorption of oxygen by green leaves in the dark and in light. ○ H_2O; □ NaN_3.

Similar inhibition ratios were obtained with long wave irradiation. The fact that the degree of inhibition of oxygen absorption by sodium azide is the same in the dark and under long wave illumination seems to indicate that the systems involved in the

References p. 152

absorption process in nonchlorophyllous leaves are the same in the dark and in the light. The inhibition was larger under short wave irradiation. This is apparently due to the fact that under these conditions activation of oxygen absorption is mainly due to the cytochrome oxidase activated by the light.

The action of sodium azide on green leaves was much different (Table IIB). Practically no inhibition of the absorption of oxygen was observed under short wave as well as long wave illumination when such poison concentrations were employed which caused an inhibition by more than 50% in the dark. Inhibition could not be induced by changing either the quality or quantity of light. An experiment carried out in the order: darkness–light–darkness (Fig. 2), showed that the inhibition which was removed in the light appeared again after the second darkening.

These results signify that the enzyme responsible for absorption of oxygen in the light is not (as in the case of non-green plants) a cytochrome oxidase of the respirative cycle.

Thus the dependence of oxygen absorption on the spectral quality of light has been found to differ in green and nonchlorophyllous plants. Different enzyme systems are involved in these reactions. Evidently these differences are due to the presence of chlorophyll. The direct dependence of the absorption of oxygen on the light intensity of long as well as short waves compels one to assume that the absorption of oxygen in green leaves takes place in the chloroplasts and is connected with a photochemical reaction excited in either of the spectral regions. The enzyme involved in this reaction may be analogous to the chloroplast "photooxidase" which is also insensitive to sodium azide[8, 10].

Some indirect evidence indicates that this photooxidase is a component of the cytochrome system located in the photoactive chloroplast complex. When long wave light is incident these systems may become activated as a result of energy migration from chlorophyll to these systems[11,12].

REFERENCES

1 R. WEINTRAUB, *Botan. Rev.* 10 (1944) 383.
2 E. RABINOWITCH, *Photosynthesis*, Vol. II, p. 2.
3 H. FÖCKLER, *Jahrb. wiss. Botan.*, 87 (1938) 45.
4 O. BODE, *Jahrb. wiss. Botan.*, 89 (1940) 208.
5 N. P. VOSKRESENSKAYA, *Problems of Photosynthesis*, Izd.A.N. SSSR, 1959, p. 335.
6 A. A. NICHIPOROWICH, *Proceedings of the International Conference on the Peaceful Uses of Atomic Energy*, United Nations, Vol. 12, 1956, p. 340.
7 N. P. VOSKRESENSKAYA AND G. S. GRISHINA, *Fiziol. Rastenii Akad. Nauk S.S.S.R.*, 7 (1960) 497.
8 R. H. NIEMAN AND B. VENNESLAND, *Plant Physiol.*, 34 (1959) 255.
9 R. H. NIEMAN, H. NAKAMURA AND B. VENNESLAND. *Plant Physiol.*, 34 (1956) 262.
10 N. I. BISHOP, H. NAKAMURA, J. BLOTT AND B. VENNESLAND, *Plant Physiol.*, 34 (1959) 551.
11 A. R. KRALL, *Research in photosynthesis*, Interscience, New York, 1957.
12 B. CHANCE AND M. NISHIMURA, *Proc. Natl. Acad. Sci. U.S.*, 46 (1960) 19.

PIGMENTS OF COMPOUND EYES

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Functionally, pigments of compound eyes may be classified as visual, screening, and reflecting. We have studied all three types of pigment in marine *Crustacea* and in insects only visual pigments.

Visual pigments were investigated *in vitro* after they had been extracted from dark-adapted eyes by various procedures. Our main purpose was to determine the absorption spectra in relation to the light conditions in which the animals normally live, and we have been especially interested in deep-sea crustaceans. The pigments were extracted from crustacean eyes with 2% digitonin, after previous removal of as much fat-soluble carotenoid material as possible. The difference curves obtained from absorption spectra measured on the digitonin extracts before and after bleaching with white light showed the following absorption maxima: for euphausiids *Meganyctiphanes norvegica*, 460–465 mμ; *Thysanoessa raschii*, 460–465 mμ; *Thysanopoda acutifrons*, 480 mμ; *Nematoscelis megalops*, 465 mμ; and *Stylocheiron maximum*, 470 mμ; for the sergestids *Sergestes arcticus*, 475 mμ; and *S. robustus*, 470 mμ: and for the oplophorid *Acanthephyra haeckeli*, 480 mμ. All these species live at depths to which only light with a spectral maximum at about 470–480 mμ would penetrate. Unsuccessful attempts to detect a visual pigment by this type of experiment were made on eyes of other crustacean species. In all we were succesful with 6 out of 11 euphausiids but with only 5 out of 18 decapods examined. In addition no visual pigment was detected in four experiments with the mysids *Eucopia sculpticauda* and *E. grimaldii*.

Under our conditions of bleaching for 5 min with a 6 V 30 W tungsten-filament bulb, the visual pigment of euphausiid eyes is bleached directly to retinene + opsin (Fisher and Goldie[1]) whereas that of decapod eyes is only bleached after a longer or second irradiation, indicating that a stable meta-rhodopsin is formed after the first exposure to light (Hubbard and Wald[2]). Our earlier finding (Fisher and Kon[3]) that the eyes of euphausiids contain rich concentrations of vitamin A which is absent from those of deep-sea decapods and many mysids may in some way be associated with this apparent difference in visual chemistry.

Recently methods involving chromatography on celite-calcium phosphate columns (Bowness and Wolken[4]) or precipitation by ammonium sulphate of proteins (Goldsmith[5]) have been reported successful in the separation of water-soluble visual pigments from the compound eyes of insects. In order to become familiar with these techniques before applying them to crustacean-eye extracts, we attempted to obtain a visual pigment from the eyes of the locust *Locusta migratoria migratorioides*. Using

References p. 154

the chromatographic method we separated fractions from dark-adapted eyes which bleached to give difference curves with absorption maxima at 440–445 mμ. Vitamin A was present in the bleached extracts. We were unable to obtain this pigment by the protein-precipitation method nor were we able to separate any photosensitive pigment by extraction with 2% digitonin.

The distribution and movements of screening pigments in the eyes of marine crustaceans have been studied by means of frozen sections. There was less astaxanthin in the eyes of the decapods examined than in those of euphausiids, in which we have previously demonstrated its movement along the ommatidia during light- and dark-adaptation (Fisher and Goldie[1]). The nature of the brown screening pigment in the eyes of the euphausiid *Meganyctiphanes norvegica* has been investigated and our earlier finding (Fisher and Goldie[1]) that it did not appear to be an ommochrome has to be qualified in that it is not an ommin according to the more refined separation technique of Butenandt, Biekert and Linzen[6].

Photomicrography with dark-ground illumination has been used to study the distribution of reflecting pigments in frozen sections of eyes of marine *Crustacea*.

REFERENCES

1 L. R. Fisher and E. H. Goldie, *Proc. XVth int. Congr. Zool., 1958*, Sect. VI, Paper 35, London, 1959, p.533.
2 R. Hubbard and G. Wald, *Nature*, 186 (1960) 212.
3 L. R. Fisher and S. K. Kon, *Biol. Revs. Cambridge Phil. Soc.*, 34 (1959) 1.
4 J. M. Bowness and J. J. Wolken, *J. Gen. Physiol.*, 42 (1959) 779.
5 T. H. Goldsmith, *Ann. N.Y. Acad. Sci.*, 74 (1958) 223.
6 A. Butenandt, E. Biekert and B. Linzen, *Z. physiol. Chem., Hoppe-Seyler's*, 312 (1958) 227.

DOSE-RESPONSE RELATIONSHIPS AT DIFFERENT WAVE LENGTHS IN PHOTOTROPISM OF *AVENA**

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In response to short, low intensity, unilateral light stimuli, etiolated *Avena coleoptiles* develop positive curvatures which are distinctly graded with respect to the light dosage applied. When the curvature is plotted against the logarithm of the dose, the response curve obtained is sigmoid, with a substantial log-linear portion in the mid-range (Fig. 2, p. 129). Because of the large errors inherent in the determination of very small responses, the shape of the lower end of the curve is obscure, but there is no obvious threshold. On linear plots of curvature versus dose the response curves all extrapolate back to zero.

These response curves are strikingly similar to another case of a distinctly graded light-growth response in plants, namely, the light-growth-reaction of *Phycomyces sporangiophores* in response to symmetrical stimuli. Here, too, the response curve as a log function of dose is clearly sigmoid[1,2], and no threshold phenomenon is apparent. All in all, it seems that these phenomena are log-linear functions of light dosage only in a very restricted sense.

In our early work on the action spectrum of phototropism we found that the response curves at various wave lengths ran closely parallel (Fig. 2, p. 129), differences in

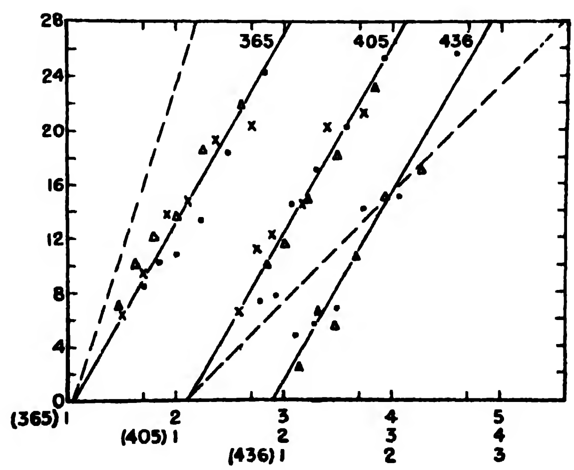

Fig. 1. Dose-reponse curves at three wave lengths. See text for details. Ordinate: curvature in degrees.

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References p. 157

wave length sensitivity being reflected only by shifts of the curves along the log-dosage abscissa. In 1958, however, Shropshire and Withrow[3] reported that not only are the curves at various wave lengths shifted along the abscissa, but also their slopes vary with wave length. This introduces variations in the action spectrum depending upon the standard response chosen for analysis. They suggested that this was due to some sort of interaction between two pigment systems, one being the photoreceptor and the other affecting the light attenuation across the coleoptile. It is not clear how this should give rise to such large variations in slope of the response curve. Others have since suggested that this constitutes evidence that two distinct photoreceptors are involved in phototropism.

Since this matter bears importantly on the question of the photoreceptor and mechanism of phototropism, we designed experiments specifically to check the slope of the dose-response curves at three critical wavelengths. The slope variations reported by Shropshire and Withrow[3] seem to us not clearly significant (Fig. 2 of ref. [3]). This is a difficult matter to settle because curvature measurements alone involve rather large errors, requiring the use of many plants for each determination. Yet the plants are subject to little understood time or age dependent variations in phototropic sensitivity, making it desirable to restrict the length of experiments as much as possible. Furthermore, red light, although it will not cause phototropic curvatures, substantially changes the phototropic sensitivity, as extensive recent work of Dr. Blaauw-Jensen has confirmed[4]. Thus, the red light commonly used to handle the plants in setting up experiments must be rigidly controlled both in respect to the intensity and duration of exposure during experiments. Finally, a response curve needs at least four points for a reasonable slope determination, and ideally two wave lengths must be quickly compared. Since the response takes 90 min or so to develop, the time dependent sensitivity variations become of serious concern. Duplicate experiments under closely identical conditions are thus exceedingly difficult to achieve. It is noteworthy that in Shropshire and Withrow's experiments only one point at a time was obtained and at least 24 h were required to determine a single response curve.

To minimize errors from all these sources we arranged a series of simple experiments in which the response curves were obtained under controlled conditions and as quickly as possible. A monochromator was placed at one end of a room having controlled temperature, humidity, and light conditions. A large number of plants were started in this room in culture plates, each containing 16 plants. Three days after the initial soaking, six plates were spaced out at measured intervals from the monochromator so that the front plate would receive approximately 50 times as high a monochromatic intensity as the back plate. The intensities at each position were determined before and after each experiment. The plates were positioned in a series of vertical steps so that there was no shadowing of one culture by another. Behind each culture, at right angles to the monochromatic beam, a sheet of green sensitive film was placed so that all the plants could be shadowgraphed with a single green exposure before and after curvature development, without having to move the plates in any way. After the first shadowgraph all the plants were exposed simultaneously to the monochromatic light for a few seconds; 100 min later a second shadowgraph was superimposed on the first to give a record of the curvatures resulting from the phototropically active light. In this manner an entire response curve was obtained in a single experiment. Then

the monochromator was set for another wave length, new plates set up, and another response curve obtained. Finally, the monochromator was reset at the original wave length and the first experiment rerun on the same day. Thus we repeatedly checked the three critical wave lengths of the easily isolated mercury lines in the blue and near ultraviolet.

The results are shown in Fig. 1. Each individual experiment at a given wave length is indicated by a series of six characters which fall roughly on a straight line when plotted logarithmically. The abscissa for each of the three wave lengths is shifted one log unit to the right to avoid overlap. The data here show triplicate runs at 365 and 405 mμ, duplicate runs at 436 mμ. We have drawn the best straight lines through all the data and have made the three solid lines parallel because the data give no indication of any difference between the slopes. The broken lines indicate the slope differences derived from the data of Shropshire and Withrow. Thus, if the slope at 405 mμ is taken from the solid line, then from their data the broken line at the far left would give their ratio of 365 to 405 slopes; conversely, if the 365 mμ slope is correct, one would have predicted the broken line at the right for the 405 mμ response curve. Our results clearly do not fit the ratios predicted by their data. We conclude that no significant difference in the slopes of the dose-response curves at various wave lengths has yet been demonstrated, and we feel that these experiments support the notion that a single photoreceptor and mechanism underlies these first-positive phototropic responses of *Avena coleoptiles*.

REFERENCES

1 M. Delbrück and W. Reichardt, in *Cellular Mechanism in Differentiation and Growth*, ed. D. Rudnick, Princeton University Press, Princeton, 19[illegible]6.

2 G. M. Curry and H. E. Gruen, *Proc. Natl. Acad. Sci., U.S.*, 45 (1959) 797.

3 W. Shropshire Jr. and R. B. Withrow, *Plant Physiol.*, 33 (1958) 360.

4 M. Blaauw-Jensen, *Thesis*, Utrecht, 1959.

PECULIARITIES OF THE OPTICAL PROPERTIES OF LEAVES DURING VEGETATION

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In the vast literature on the study of the optical properties of leaves, this question is discussed mainly from the point of view of studying light absorption, reflection and transmission in the static state. In Rabinovitch's monographs[1,2] (1951, 1956), French's review papers[3,4] (1956–1959), Kleshnin and Shulgin's papers[5,6] (1959, 1959) and the paper by Shibata[7] (1959) we see a detailed analysis of spectral curves which express the optical properties of leaves in connection with the chloroplast pigment system, with the structural peculiarities of leaves, which refer to different ecologic groups and so on. Until now the problem of the changes of the optical properties of leaves during the vegetation period has been studied insufficiently.

In the present investigation we directed our efforts to the study of the optical properties of leaves from two silvan species: (1) light plants *Betula verrucosa* and (2) shade plants *Tilia cordata*, growing under natural conditions near Moscow. Systematic measurements of the leaves' optical properties, that is, the coefficient of light absorption, reflection and transmission have been done with the help of an integrating photometric sphere (Tageeva[8]); simultaneously in parallel, the chlorophyll and carotenoid content of control leaves, as well as their weight and area were determined. The first determinations were done on May 13, approximately 7–10 days after the beginning of blossom, and lasted during the whole summer period, until the leaves grew yellow – October 1, the end of leaf existence.

In Fig. 1 the data for *Betula* are represented. Curves 1, 2, and 3 show changes of absorption coefficients during the leaf existence in three regions of the spectra. We can see that absorption of light by a leaf in the blue region of the spectra (curve 1) remains on the same level (95%) during almost the whole vegetation period, decreasing insignificantly at the end of vegetation to 92%. The absorption coefficient in red (curve 2) and green rays (curve 3) changes much more. It can be noted that the absorption coefficients in the red and green regions grow considerably: from 65 to 85% and from 55% to 78% respectively, increasing with a more intensive chlorophyll formation. After reaching this level of light absorption in the red and green regions in the beginning of June, the spectral coefficients of absorption remain almost constant during the main period of vegetation until the middle of September. The amount of chlorophyll of 2–2.5 mg/100 cm^2 is optimal and provides for the maximal light absorption by a leaf in different regions of the spectra. Further change of the chlorophyll

content in the fully grown and shaped leaves is not followed by the change of the light absorption coefficient, which reaches the plateau and remains there until the autumn period, as can be seen from Fig. 1. But the absorption in the red and green regions falls very sharply at the end of vegetation, when the chlorophyll content decreases and becomes less than 2 mg/100 cm².

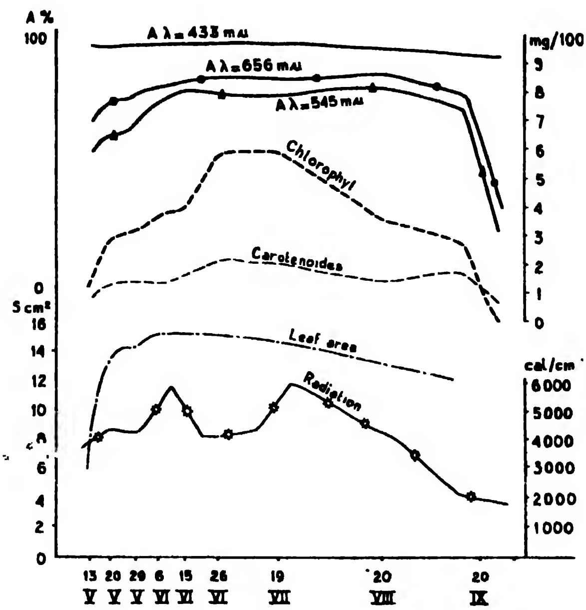

Fig. 1. *Betula verrucosum*. Dynamics of spectral coefficients of light absorption by a leaf and pigment content during vegetation period.

Variations in the intensity of radiation during the season do not change the absorption coefficient; its value does not depend on radiation intensity As is evident from the experiments, the change of radiation from 19/VII to 20/IX is not followed by the simultaneous decrease of the light absorption coefficient; at the same time a sharp decrease of the pigment content from 20/IX to I/X called forth correspondingly almost complete cessation of light absorption in the red and green regions of the spectra. The light absorption is defined not only by pigment systems but also by structural elements, such as a thicker leaf structure due to the presence of thick membranes of cells and a greater amount of mechanical tissues. As can be seen from curve I, which characterises the coefficient of absorption in the blue region of spectra, the coefficient did not alter during an autumn period, when considerable destruction of pigments took place.

On Fig. 2 can be seen the data for a shade plant of *Tilia cordata*. The direction of the curves reminds one very much of the already discussed data for *Betula*. However, there are certain differences, which are connected with the structural peculiarities of the constitution of a thinner leaf of *Tilia* which contains fewer mechanical elements and thinner mesophyll than in the leaf of *Betula*. Therefore there is a closer similarity between the curves of absorption coefficient and chlorophyll content in *Tilia* leaf in all spectral regions.

References p. 162

It is very interesting to compare the irradiation data for *Tilia* and *Betula*. In forest conditions, where the *Tilia* trees taken for our experiments grew, irradiation did not exceed 600–1,000 cal/cm², and in the forest with *Betula* it varied during the summer period between 4,000–6,000 cal/cm² for 10-day period. Thus, the leaves of *Betula*

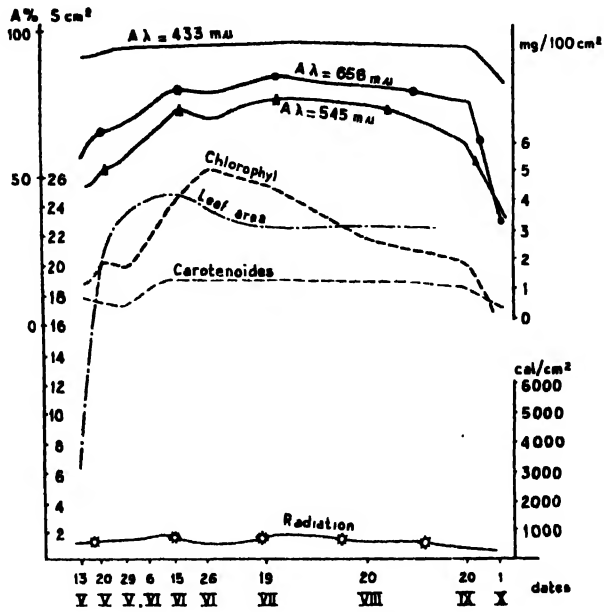

Fig. 2. *Tilia cordata*. Dynamics of spectral coefficients of light absorption by a leaf and pigment content during vegetation period.

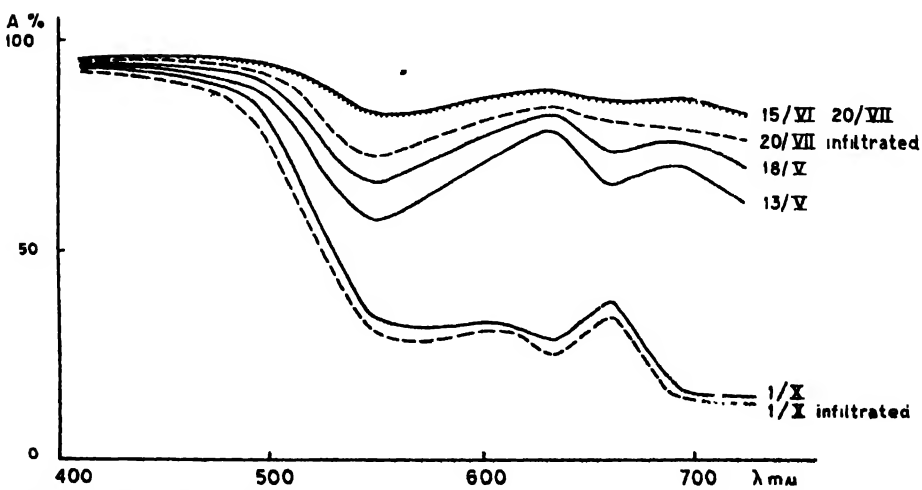

Fig. 3. *Betula verrucosum*. Spectral coefficient of light absorption in normal and infiltrated leaves during various vegetation periods.

received 6 times more radiant energy than *Tilia*. However the coefficients of absorption for both species were practically equal. Our experiments illustrate very well the statement about more effective use of radiant energy by those plants which are

adapted during their long evolution to the condition of insufficient illumination. It is *Tilia* which compensates for light deficiency by its intensive growth of foliage area. The area of one average leaf of *Tilia* is almost two-times larger than a *Betula* leaf.

This makes it possible to absorb more completely all the radiant energy incident on the tree. It also results in the fact that accumulation of weight in one leaf of *Tilia* is only two times less than the weight of *Betula*. The weight of *Tilia* leaf (mg/cm²) equals 8 mg/cm² and in *Betula* 14 mg/cm².

This is proved by the comparison of our data plotted according to the spectral distribution of coefficients of light absorption by the leaves in different periods of vegetation.

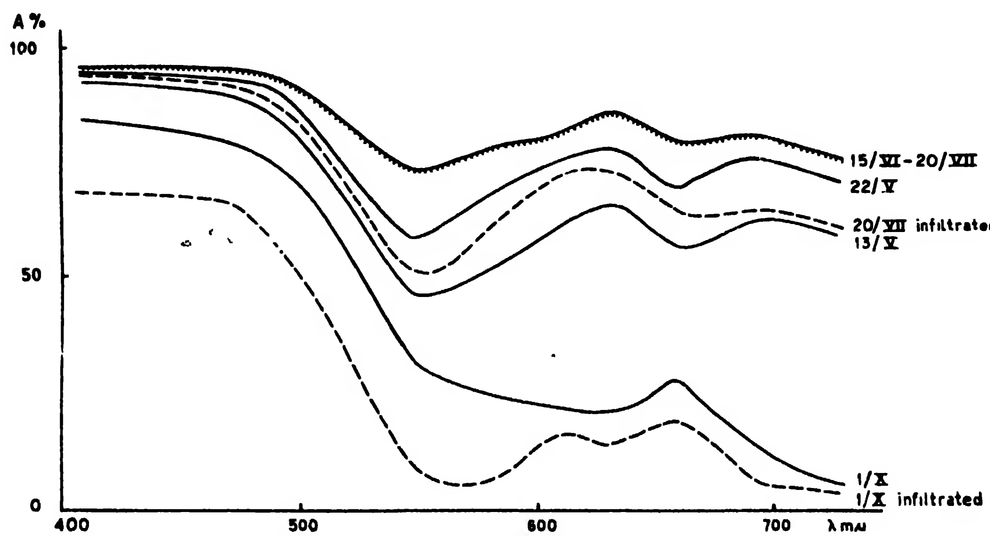

Fig. 4. *Tilia cordata*. Spectral coefficients of light absorption in normal and infiltrated leaves during various vegetation periods.

From Fig. 3 can be seen how quickly the optic apparatus is formed in a young, intensively growing leaf. Approximately 10 days after the beginning of leaf growth, the capacity for light absorption in the blue and even red regions of the spectra reaches maximal values. Infiltration of a thicker xeromorphic leaf of *Betula* with water does not significantly decrease the absorption coefficient. After the infiltration we observed virtually no changes in the red, green or the blue regions.

The curves of the experiments carried on with yellow leaves of *Betula* on October 1, represent sharp violation of the absorption in the region 520–700 mμ. In this case, infiltration of *Betula* leaf also did not bring about any considerable changes. As far as the similar data for *Tilia* (Fig. 4) are concerned, there are, together with general similarity, considerable differences from the curves for *Betula*, connected with peculiarities of constitutions of the thinner leaf of *Tilia*.

Thus, infiltration in both experiments (on June 20 in the period of the leaf's most intensive activity, and in the period of autumn leaf withering) leads to a considerable

References p. 162

decrease of light absorption, mainly in the green region of the spectra with less absorbing power and a slightly smaller decrease in the red region.

Infiltration of leaves and filling the air-containing spaces decreases the heterogeneity of their internal structure and changes the relation of the optical properties. From this it is evident that intracellular spaces in leaf mesophyll play an important role not only as air-containers but help to multiply reflection of light inside the leaf which increases its absorbing abilities and utilization by the plant.

REFERENCES

1 E. RABINOVITCH, *Photosynthesis and Related Processes*, Vol. 2, Part 1, Chapter 22, 1951, p. 672
2 E. RABINOVITCH, *Photosynthesis and Related Processes*, Vol. 2, Part 2, Addendum Chapter 22 1956, p. 1841.
3 C. FRENCH, *Radiation Biology*, Vol. 3, Chapter 6, 1956, p. 343.
4 C. FRENCH, *Photoperiodism and Related Phenomena in Plants and Animals*, 1959.
5 A. KLESHNIN AND I. SHULGIN, *Doklady Akad. Nauk S.S.S.R.*, 125 [5] (1959) 1158.
6 I. SHULGIN AND A. KLESHNIN, *Doklady Akad. Nauk S.S.S.R.*, 125 [6] (1959) 1371.
7 K. SHIBATA, *Methods of Biochem. Anal.*, 7 (1959) 77.
8 S. TAGEEVA AND A. BRANDT, *Biofizika*, 4 [2] (1959) 232.

STUDY OF OPTICAL PROPERTIES OF LEAVES DEPENDING ON THE ANGLE OF LIGHT INCIDENCE

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Illumination of plant leaves by the rays of the sun during daytime occurs with ever-changing incidence angle, which is determined by the height of the sun over the horizon. Therefore investigation of the problem of the extent to which the optical properties, leaf absorption of light in particular, can depend on the change of the angle of ray incidence angle, is of special interest.

The leaf is a heterogenous system with a highly complicated structural organisation and with different optical density of the component elements such as: cell membrane, protoplasm, plastids containing various pigments, stained cell sap, the dense network of intracellular spaces filled with air, water vapours.

The rays of light, encountering such a heterogenous medium, undergo multiple refraction, reflecting at different angles which leads to considerable light scattering and its secondary absorption inside the leaf. Therefore, it is impossible to calculate the change of absorption, reflection and transmission coefficients, with the change of angle of incidence of the irradiating light in accordance with the law of geometrical optics.

There are certain papers in the literature, which describe the problem of the leaves'

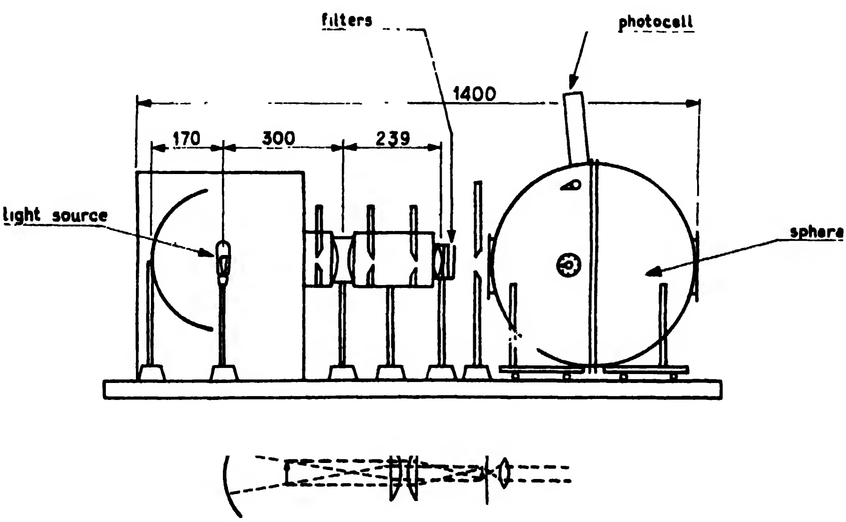

Fig. 1. Universal arrangement for the study of optical properties of leaves.

References p. 169

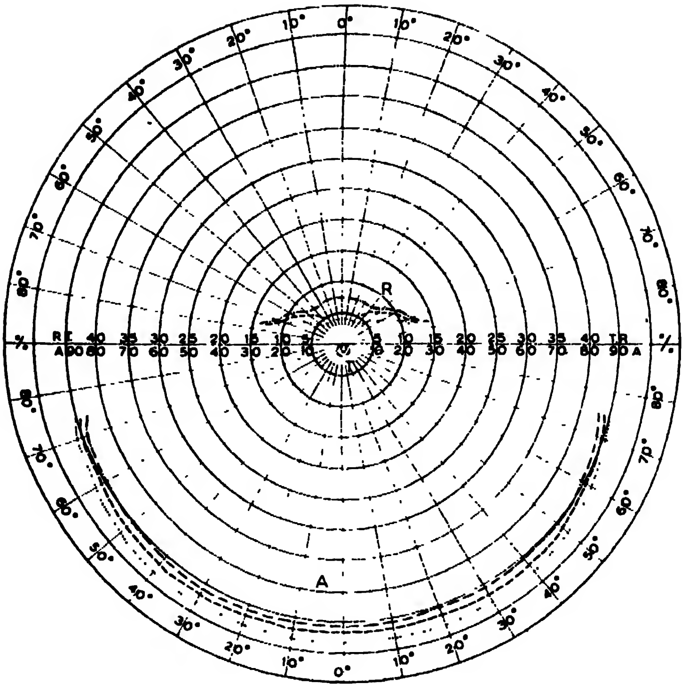

Fig. 2. Spectral coefficient of reflection (R), transmission (T), and absorption (A) of light by a leaf of *Ficus elastica* depending on the change of light incidence angle. — λ = 656 mμ; —·· ·—· – λ = 545 mμ; ...... λ – 463 mμ.

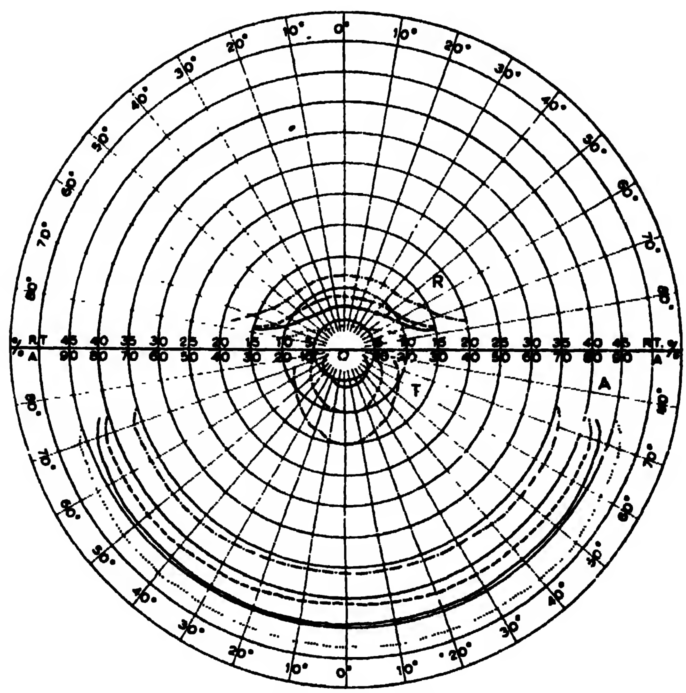

Fig. 3. Spectral coefficients of reflection (R), transmission (T) and absorption (A) of light by a leaf of *Hibiscus rosa sinensis* depending on the change of light incidence angle. · — — · white light; —·—·—·— λ = 545 mμ; ---- λ = 656 mμ; ...... λ = 463 mμ.

scattering ability by the nature of the indicatrissae of scatter of the reflected and transmitted light with a constant incidence angle (Seybold[1], Loomis *et al.*[2], Rabinovitch[3]). But a biologist is much more interested in studying the light absorbed by the leaf which calls forth all the physiological processes in living plants depending on a different angle of light incidence. Therefore the present investigation is a study of absorbtion coefficients of different regions of the spectra, depending on the change of the light incidence angle. Simultaneously the light reflection and transmission coefficients have been studied. The measurements have been done on a universal arrangement for the study of leaf optical properties, proposed by the authors (Tageeva and Brandt[4]) in white light from a cinema lamp (500 W), and in a monochromatic light obtained with the help of interference light filters of λ = 404, 463, 545 and 656 mμ.

As is seen from Fig. 1 our arrangement with an integrating photometric sphere d = 500 mm, enables us to place an intact leaf inside the sphere and to change the position of the leaf plane in relation to the ray of the incident light by means of a special device. This arrangement also enables us to evaluate the leaf optical properties depending on the change of the light incidence angle.

Leaves with different structure were used for investigation. thick glossy leaves *Ficus elastica* (Fig. 2) less thick leaves with glossy surface *Hibiscus rosa sinensis* (Fig. 3) and the leaf of *Lactuca sativa* with a thin light plate (Fig. 4). Besides, it was interesting to study the leaves of *Coleus* (Fig. 5), which contain anthocyans in the cell sap of the upper epidermis.

To consider the role of one or another structural element of the leaf in the light absorption and scattering, in addition to the study of common leaves, we used on the one hand, leaves infiltrated by water which considerably decreased their heterogeneity while the pigment apparatus was preserved, and on the other hand yellow autumn leaves with constant heterogeneity, but a destroyed pigment composition.

From the given Figs. 2–5 it can be clearly seen that the reflection coefficient of light changes greatly with the change of the light incident angle. Thus at the incident angle 0°, that is with the incident light perpendicular to the plane of a leaf, the reflection coefficient is minimal, and with increase of the incident angle it increases greatly. Similar phenomena can be observed both in thin dull leaves (Fig. 4) and in leaves with glossy surfaces (Fig. 2). Another regularity is observed in the study of the transmission coefficient of light by the leaves. On perpendicular illumination the transmission coefficient is maximal and it becomes minimal on illumination at an angle of 75°.

Owing to the fact that with the increase of the reflection coefficient a simultaneous decrease of the transmission coefficient is observed along with a simultaneous change of the incidence angle from 0° to 55°, the coefficient of absorption of light with different wave lengths remains practically constant. It is only at the angles of incidence more than 60° that the absorption coefficient decreases insignificantly.

The experiments with a leaf of *Coleus* (Fig. 5) show, that due to the supplementary pigment (anthocyans), the coefficient of absorption increases greatly and, correspondingly, coefficients of transmission and reflection decrease, but the shape of the curves of optical parameters remains, as in the common green leaves, dependent on the angle of light incidence.

We think that on the basis of the adduced experimental data it is impossible to draw any conclusions about the ideal scattering ability of leaves since coefficients of

References p. 169

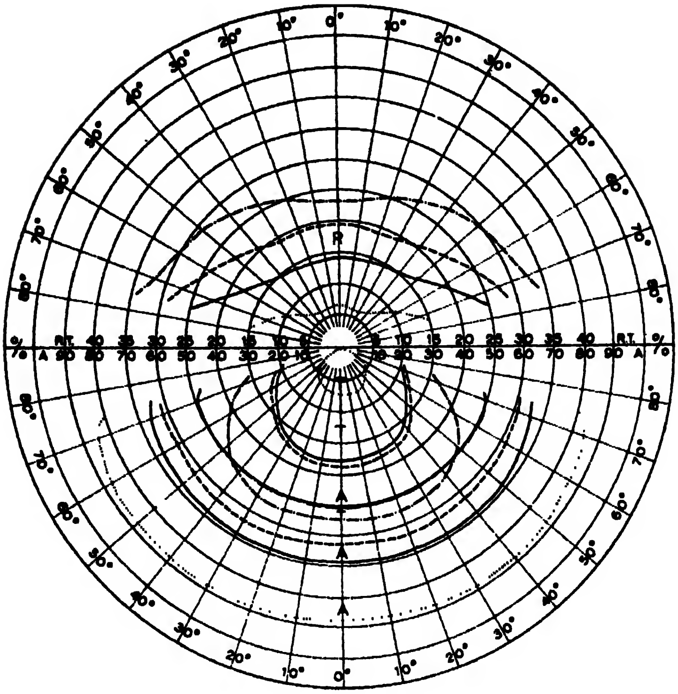

Fig. 4. Spectral coefficients of reflection (*R*), transmission (*T*) and absorption (*A*) of light by a leaf of *Lactuca sativa* depending on the change of light incidence angle. - ——— white light; - - - - $\lambda = 656$ mμ; - · — · —·- $\lambda = 545$ mμ; . . . . . . $\lambda = 463$ mμ.

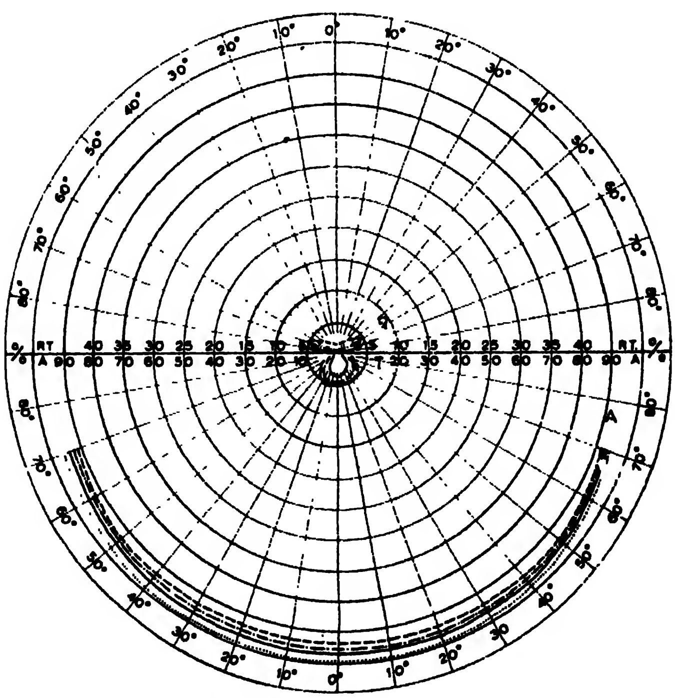

Fig. 5. Spectral coefficients of reflection (*R*), transmission (*T*) and absorption (*A*) of light by a leaf of *Coleus* depending on the change of light incidence angle. ——— white light; - - - - - $\lambda = 656$ mμ; —· — —· —— $\lambda = 545$ mμ; . . . . . . $\lambda = 463$ mμ.

transmission and reflection of light by the leaf change considerably depending on the angle of light incidence. We can define an ideally scattering medium as one in which transmission and reflection of light satisfies Lambert's law, and the brightness is equal in all directions no matter what is the direction of light falling on the irradiating plane.

Now let us consider the data from the study of infiltrated leaves. The optical heterogeneity of the leaf tissue structure conditioned by a great number of surfaces of separation between the solid, liquid and gaseous phases determines its complicated optical properties as those of a dull, intensively scattering medium. Therefore with the change in this heterogeneity, for example, with its weakening at the expense of air displacement from the intercellular spaces and the substitution of the gaseous phase by water, we must weaken the scattering ability of a leaf tissue and change its optical parameters.

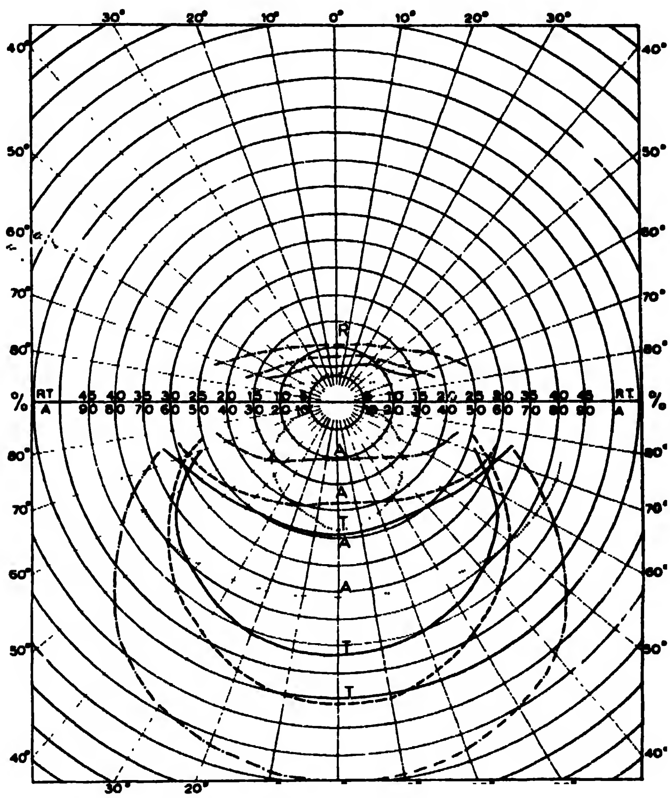

Fig. 6. Spectral coefficients of reflection (R), transmission (T) and absorption (A) of light by an infiltrated leaf of *Lactuca sativa* depending on the change of light incidence angle. ——— white light; - - - - $\lambda = 656$ mμ; —·—·—·— $\lambda = 545$ mμ; ...... $\lambda = 403$ mμ.

The results of measurements are given in Fig 6 (*Lactuca*) and Fig. 7 (*Hibiscus*). In fact it is possible to note a considerable decrease of light reflection coefficient in infiltrated leaves at the expense of a decrease of the back reflection on the border of two phases (cell membrane, water in the intracellular spaces). However, the general regularity of the shape of the curves in the reflection coefficient of the incident light remains as in the control leaves, that is, with increase of the incidence angle the reflection coefficient increases.

References p. 169

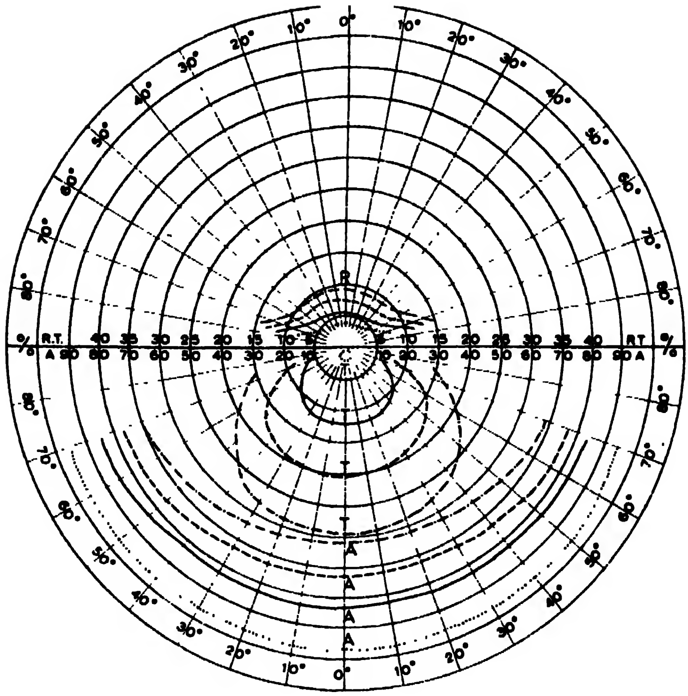

Fig. 7. Spectral coefficients of reflection (R), transmission (T) and absorption (A) of light by a infiltrated leaf of *Hibiscus rosa sinensis* depending on the change of light incidence angle. —— white light; - - - - λ = 656 mμ; · - · -— λ = 545 mμ; . . . . . . λ = 463 mμ.

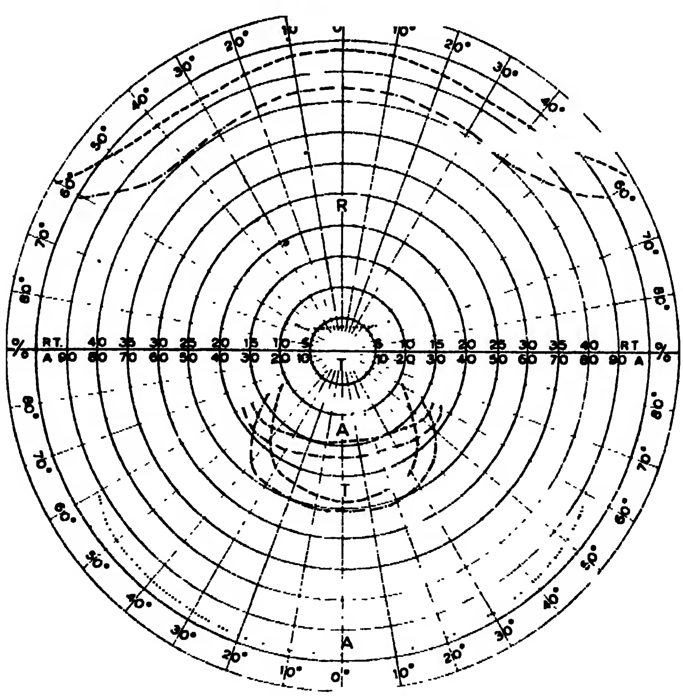

Fig. 8. Spectral coefficients of reflection (R), transmission (T) and absorption (A) by a yellow leaf of *Ficus elastica* depending on the change of light incidence angle. - - - - λ = 656 mμ; —·—· - · - λ = 545 mμ; . . . . . . λ = 463 mμ.

The transmission coefficient increases greatly upon leaf infiltration but it decreases, as in the control leaf, with the increase of the light incidence angle.

The greatest deviations from the norm are observed on the absorption of light by the thin leaves of *Lactuca* (Fig. 6) after their infiltration. With the increase of the angle of light incidence on the leaf, the absorption coefficient increases greatly, compared with the control leaves, it being known that the degree of increase depends on the spectral composition of the incident light. Thus, for the ray $\lambda = 545$ mμ, the absorption coefficient increases from 21% (in the control) to 44% in infiltrated leaves on the change of the incidence angle from 0° to 75°, and for the rays of $\lambda = 463$ mμ the absorption increase goes on correspondingly from 71% only up to 84%.

This increase of the coefficient of absorption of light by the leaf with the increase of incidence angle in the infiltrated leaves can be explained by the decreases of the leaf heterogeneity and thus the less intensive light scattering, on the displacement of air in the intracellular spaces by water. This results in the fact that the distance of light passage through the leaf lengthens, while the absorption increases proportionally as well.

However, absolute values of the absorption coefficients in infiltrated leaves will be less than in the control at all the angles of light incidence.

Our data on the comparative study of the optical properties of the infiltrated and common leaves allow us to conclude that the intracellular spaces in the leaf mesophyll not only are the system in which a leaf gaseous exchange is fulfilled, but also assist in a greater internal scattering of the light penetrating into a leaf and provide for a better light utilization on the repeated internal reflextions. Although the geometrical optics of the leaf are very important for the description of its optical properties, the system of pigments which actively absorb luminous energy is of a primary importance.

A convincing support of this statement is the data given below on the evaluation of optical parameters in leaves that became yellow and contained sharply decreased amount of chlorophyll. Thus in a yellow leaf *Ficus* (Fig. 8) compared with a common green leaf (Fig. 1), coefficients of reflection and transmission sharply increase in the rays $\lambda = 545$ mμ and 656 mμ while in the rays $\lambda = 463$ mμ there are almost no changes. Simultaneously, the absorption coefficient abruptly falls in the rays $\lambda = 545$ mμ and 656 mμ and remains almost constant in the rays $\lambda = 463$ mμ.

As far as the light reflection, transmission and absorption coefficients are concerned in the leaves that had yellowed, depending on the angle of light incidence the shape of their curves remains as in the common leaves.

Due to the fact that the maximal height of the sun over the horizon is at 50–55° in the mean latitudes during the day in summer, and the maximal irradiation has a surface perpendicular to the incident rays (incidence angle 0°) the position of the majority of leaves at the angle 45–55° to the horizon becomes clear. The leaves are thus in the most favourable position (to the direction of incident rays), for the greater part of the day light period, when the absorption coefficient is the greatest.

REFERENCES

1 A. Seybold, *Planta*, 20 (1933) 577.
2 W. Loomis, C. H. Randall and G. Island, *A.A.A.S. Symposium on Photosynthesis*, 1941.
3 E. Rabinovitch, *Photosynthesis and Related Processes*, Vol. 2, Part 1, 1951, p. 672.
4 S. Tageeva and A. Brandt, *Biofizica*, 4 [2] (1959) 232.

STUDIES OF THE FUNCTION OF PHOTOSYNTHETIC PIGMENTS

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A number of studies in recent years have indicated that activation of more than one pigment may be necessary for efficient photosynthesis[1-7]. The pigments may be chlorophylls *a* and *b*, chlorophyll *a* and a biliprotein pigment, chlorophyll *a* and a photosynthetically active carotenoid, or even two *in vivo* "forms" of chlorophyll *a*[5-7]. The experiments supporting this concept depend upon increases in photosynthesis with a given wavelength of monochromatic light when a second wavelength, absorbed by a second pigment, is added, or upon observation of transients on passing from photosynthesis at one wavelength of light to that at another. The recent studies of Myers and French[7], in which it was shown that the different wavelengths of light exciting the two pigments produce their effect even when applied several seconds apart, have suggested the possibility of formation of different photoproducts in reactions catalyzed by the different pigments.

Since it seemed *a priori* likely that free radicals might be among the photoproducts, the production of these by light of various wavelengths has been investigated by electron paramagnetic reasonance (EPR) spectroscopy. Production of free radicals upon illumination of chloroplasts or algae has previously been observed by Commoner *et al.*[8,9] and by Calvin and associates[10,11], but the strong absorption of microwaves by liquid water forced these investigators to work with thick pastes of material under unphysiological conditions. Development by Varian Associates of a thin quartz cell which can be positioned in the nodal plane of the microwave cavity has made it possible to work with cell suspensions under physiological conditions.

On illumination of *Chlorella pyrenoidosa* with white light an EPR signal with half band width of 14 gauss was induced. No fine structure of the band could be detected. On darkening, a portion of the signal decayed rapidly, while the remainder decayed very slowly, taking up to 5 min to disappear. The rise and decay times of the "fast" signal depended upon the density of the cell suspension used and on whether it had been recently stirred.

Illumination of a non-photosynthetic *Chlorella* mutant induced only a trace of microwave absorption.

The blue-green alga *Anabaena cylindrica* and the non-sulfur purple bacterium *Rhodopseudomonas palustris* both gave on illumination a single sharp signal with half band width of 10 gauss. The rise and decay times of this signal were around 1/3 sec;

* Supported in part by contract AT (04-3)-232 with the U.S. Atomic Energy Commission.

no slowly decaying component was observed. The band widths of the signals agree in general with those observed previously[10,11], but the rise and decay times are different, probably due to the difference in experimental conditions.

Addition of potassium cyanide (0.01 *M*) to a suspension of *Chlorella pyrenoidosa* completely abolished the slowly decaying component of the signal; addition of sodium azide or hydroxylamine reduced its magnitude and hastened its decay. None of these inhibitors affected the fast component.

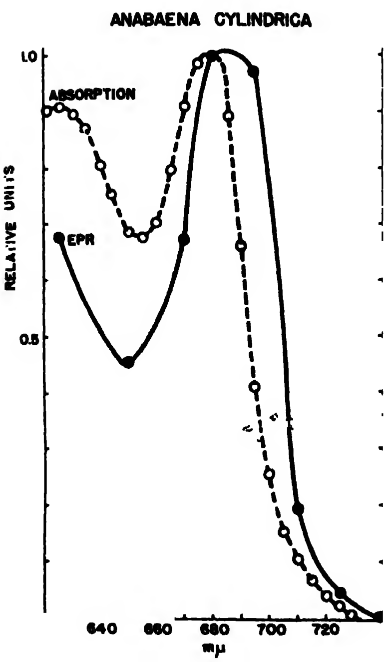

Fig. 1. Comparison of action spectrum for induction of EPR signal in *Anabaena cylindrica* with the absorption spectrum of the alga.

The EPR signals produced in all three organisms by monochromatic light were similar to those induced by white light. The action spectrum for excitation of the EPR signal in *Anabaena cylindrica* is shown in Fig. 1. It will be seen that it follows in general the absorption spectrum of the cells, but that the long wavelength region of the chlorophyll absorption band, which is relatively inefficient for photosynthesis, induces a greater EPR signal than expected, whereas phycocyanin absorbed light, which is photosynthetically effective, gives a smaller EPR signal than expected from the absorption. Since it is the steady state concentration of free radicals that is measured in these experiments, it seems possible that their more rapid removal under optimal photosynthetic illumination, and their less rapid removal under sub-optimal, can account for this variation. The action spectrum for excitation of the fast signal in *Chlorella pyrenoidosa* also follows the general pattern of the absorption spectrum of the cells, but shows a broadening which may well have the same cause as discussed for *Anabaena*. It will be of great interest to observe the effect on the EPR action spectrum of addition of a second wavelength of light.

REFERENCES

1 R. Emerson, *Science*, 127 (1958) 1059.
2 R. Emerson and R. F. Chalmers, *Phycol. Soc. Am. News Bull.*, 11 (1958) 51.
3 L. R. Blinks, in H. Gaffron, (ed.), *Research in Photosynthesis*, Interscience, New York, 1957, pp. 444–449.
4 L. R. Blinks, *Plant Physiol.*, 34 (1959) 200.
5 L. R. Blinks, in M. B. Allen (ed.), *Comparative Biochemistry of Photoreactive Systems*, Academic Press, New York, pp. 367–376.
6 C. S. French, J. Myers and G. C. McLeod, in M. B. Allen (ed.), *Comparative Biochemistry of Photoreactive Systems*, Academic Press, New York, pp. 361–366.
7 C. S. French and J. Myers, *J. Gen. Physiol.*, 43 (1960) 723.
8 B. Commoner, J. J. Heise and J. Townsend, *Proc. Natl. Acad. Sci. U.S.*, 42 (1956) 710.
9 B. Commoner, J. J. Heise, B. B. Lippincott, R. E. Norberg, J. V. Passonneau and J. Townsend, *Science*, 126 (1957) 57.
10 G. Tollin, P. B. Sogo and M. Calvin, *Ann. N.Y. Acad. Sci.*, 74 (1958) 310.
11 P. Sogo, M. Jost and M. Calvin, *Radiation Research, Suppl.*, 1 (1959) 511.

ABOUT CERTAIN PECULIARITIES OF BIOLOGICAL ACTION OF U.V. RAYS WITH DIFFERENT WAVE LENGTH

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The present investigation is a study of the effect of U.V. rays of different wave length on the skin electrical conductivity, *erythemal* reaction and white corpuscles of rats. It is evident that the cause of different effects of U.V. rays lies in the specificities of photochemical reactions. This difference consists not only in the fact that one region of the spectra affects proteins and other materials such as nucleic acids, but also in the fact that different regions of the spectra bring about different changes in one and the same irradiated substrate. Thus Kofman (1946) in *in vitro* experiments showed that short wave rays bring about primarily coagulation and denaturation of proteins, whereas long wave rays cause processes of photolysis. It is probable that general regularities in the effect of U.V. rays with different wave length which were observed in the *in vitro* experiments can also appear in the whole organism.

It is known that on irradiation with U.V. rays the permeability of cells with respect to organic and inorganic substances changes, which results in redistribution of ions between cells and intracellular media and pH changes in subcutaneous cellular tissue. For the study of ion redistribution at present a technique of electrical conductivity is successfully used. This technique permits the study of physico-chemical processes in tissues without disturbing their life activity.

We expected that this technique would help us to detect differences in the effect of U.V. rays of different wave length on the physico-chemical state of skin, and also to detect the phenomena that are connected with its functional state, for example, the filling of the vessels with blood.

Skin electrical conductivity was measured at two frequencies, *viz* 10^6 and 10^4 Herz. As far back as 1940 Tarusov proposed that the index of viability of tissues should be considered not as the absolute values of resistance but as the ratio of low-frequency resistance (R_4) to high-frequency resistance (R_6). R_4/R_6 is termed the coefficient of polarization. The following sources of U.V. radiation were used: sunburn lamps (U.V. *A*, max 365 mμ), *erythemal* lamps (U.V. *B*, max 310 mμ) and bactericidal lamps (U.V. *C*, max 254 mμ). Irradiation was done with different doses.

The data obtained show that the coefficient of polarization decreases on U.V. radiation, mainly at the expense of low-frequency resistance. Dependence of the skin reaction on the length of the irradiation wave is observed: on irradiation with U.V. *C* (254 mμ) the coefficient of polarization begins to decrease immediately after irradiation even before the appearance of *erythema*, on irradiation with U.V. *B* (310 mμ) only several hours afterwards, and in many cases only after the appearance of

erythema. On irradiation with U.V. *A* (365 mμ) *erythema* did not appear and the coefficient of polarization does not change. In all experiments the coefficient of polarization decreases more intensively, the brighter the *erythema*, and as a rule the maximal *erythema* is always followed by a minimal value of the polarization coefficient; with the disappearance of *erythema* the polarization coefficient returns to the norm.

With strong *erythema*, the polarization coefficient decreases more than 8-fold. Thus, it becomes evident that the change of the coefficient of polarization is defined by the *erythemal* reaction; this is especially clearly seen for U.V. *B*. But in the initial period after irradiation with U.V. *C*, the polarization coefficient is independent of the development of the *erythemal* reaction; this is the principal difference between the two comparable areas of the spectra. It can be supposed that during the first hours after irradiation the specificity of the changes of the polarization coefficient depends on the peculiarities of the physico-chemical processes which appear in tissues depending on the length of irradiation wave, but further changes of the polarization coefficient are defined also by the *erythemal* reaction.

Erythema and skin electrical conductivity are the indices of local organism reaction. White blood corpuscles were studied as an index of general reaction. The amount of leucocytes increases on irradiation but the dependence of the blood reaction on the wave length of irradiation was also observed. After treatment by U.V. rays (U.V. *C*) as a rule one maximum in the amount of leucocytes appears in time; on irradiation by U.V. *B* and *A* in the majority of experiments two maxima appear.

In the course of this investigation the intensity of local (*erythema* and blood electrical conductivity of the skin) and general (blood) reactions of the organism were compared. The results have proved that in half of the 60 experiments carried out, the degrees of general and local reactions of the organism do not correspond; that is, with strong *erythema* the blood reaction does not change at all, and if the polarization coefficient does not change, a more than three-fold increase in the leucocytes amount takes place. Thus, *erythema* is rather an index of the light-sensitivity of the organism, rather than an index of its functional state.

The data obtained prove that U.V. rays of different wave length have different effects on local phenomena (*erythema*, blood electrical conductivity) and general phenomena (blood). The specificity of the effect of light of different wave length can be partially detected with the help of the method of electrical conductivity used in the present investigation.

BIOLOGICAL ACTIVITY OF THE PHOTOSYNTHETIC PRODUCTS OF PLANTS ILLUMINATED IN LIGHT OF VARIOUS SPECTRAL COMPOSITION

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Extensive data now available indicate that even after very brief illumination of plants, the radioactive carbon absorbed by them during photosynthesis can be detected in a variety of substances. We have found that the composition and relative quantities of labelled products can change depending on the conditions prevailing during photosynthesis[1,2]. On this basis we[1,2] have repeatedly suggested that the biochemical specificity of the plants, which determines many peculiarities of their physiological condition or of their physiological response to the environment, stems from qualitative differences in the products of photosynthesis formed under various conditions. However, a purely chemical analysis of the photosynthetic products would not be sufficient to determine the physiological significance of the various products formed under different conditions.

In an attempt to solve this problem we have performed some experiments making use of biological tests. The composition of the products of photosynthesis was changed, for example, by illuminating the plants with light of differing spectral composition. This factor, which has been studied in some detail[1-7] pronouncedly affects the chemical mechanisms of the plants and hence the composition of the products and the growth and development processes.

Bean plants (variety "Triumf sakharnaya" N 764) were grown for 14 days in light from white light fluorescent lamps (w.l.) (experiment variant 2). After 24 h in the dark they were illuminated for 3 h in closed compartments with light from: (*1*) red fluorescent lamps, phosphor $ZnBiSiO_3$ (variants 6 and 7); (*2*) white fluorescent lamps (w.l.) (variants 8 and 9) and (*3*) blue fluorescent lamps, phosphor $CaWO_4+MgWO_4$ (variants 10 and 11). The irradiance under the blue lamps was 24.400 erg/cm²/sec, under the white lamps 20.000 erg/cm²/sec and under the red lamps 19.380 erg/cm²/sec which corresponds to equal fluxes of quanta (in "einsteins"). In each of the indicated types of experiment there were two variants, — one in which the illumination was carried out in the presence of CO_2 (0.04 vol.%) and one without CO_2.

As controls some experiments were carried out by cultivating the plants over a prolonged period (var. 1 and 2) in red or blue light or by keeping the plants in the dark in the presence or absence of CO_2 (var. 4 and 5).

The complete scheme of the experiment was thus as follows:

(a) Plants grown 14 days in

(1) red light	(2) white light	(3) blue light

(b) 24 h darkness

(c) 3 h exposure of plants in

darkness		red light		white light		blue light	
$+CO_2$	$-CO_2$	$+CO_2$	$-CO_2$	$+CO_2$	$-CO_2$	$+CO_2$	$-CO_2$
(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)

After exposure the leaves were detached and after removal of the major veins, were fixed in liquid nitrogen, ground in the frozen state and extracted with water over 18 h at a temperature of about 0°. The extract from 4 g of fresh leaves was added to one litre of the nutrient medium in which the tissue of the test object was grown. As test object we employed a carrot root tissue culture obtained in 1956 from M. Zelinski of Poznan University to whom we would like to express our appreciation. In 1957 and later the tissue was cultivated in Nitsch's agar medium[8] which besides mineral salts includes 25 g/l of glucose. The tissue used in the experiments belongs to the habituated type and does not require the addition of auxins to the nutrient medium for growth. It is moreover autotrophic in respect to vitamin synthesis and hence it was not necessary to add vitamins to the medium.

A priori, therefore, it could be assumed that if any active substances were found in the extracts which caused a positive or negative response of the tissue, the probability that these substances were carbohydrates, auxins or vitamins would be very low provided, of course, that the amount of these substances did not increase to abnormal values. The leaf extracts were added to the medium prior to its sterilization in the autoclave (at 0.75 atm for 20 min). The tissue was cultivated in the dark in an air conditioned room at 26° and relative humidity of 70% for a period of 7–8 weeks. In each experiment there were 10 pieces of root tissue. After growth of the tissues they were weighed and the percent of dry weight was determined in each of the variants. The results of such measurements are shown in Figs. 2–7.

The columns of Figs. 2–7 designate the weight in mg of each of the 10 calluses in each variant of the experiment.

It will be seen that the extract from leaves exposed to light under various conditions possesses different physiological activity. Thus that from leaves of plants grown for 14 days in blue light (variant 3), or exposed for 3 h to blue light (variant 10), stimulates the growth of carrot tissues to a much greater extent than does the extract from the leaves of plants grown in (variant 1), or exposed to (variant 6), the light of red lamps. With respect to biological activity, the extract from leaves of plants grown under white light lamps (variant 2) occupies an intermediate position. However, the most important result of these experiments is the fact that photosynthesis is the decisive factor determining the biological activity of the extracts of plant leaves exposed to red or blue light. From Figs. 4 and 5 it can be seen that exclusion of CO_2, that is, prevention of photosynthesis during exposure of the plant in blue light (variant 2) considerably lowers the stimulating action of the extract from these leaves. On the other hand,

prevention of photosynthesis in plants exposed to red light (variant 7) considerably enhances the stimulating action of the resulting extract . Exposure of the plants in white light in the presence (variant 8) or absence (variant 9) of CO_2 yields results which are similar, insofar as the plant leaf extract activity is concerned, to those obtained

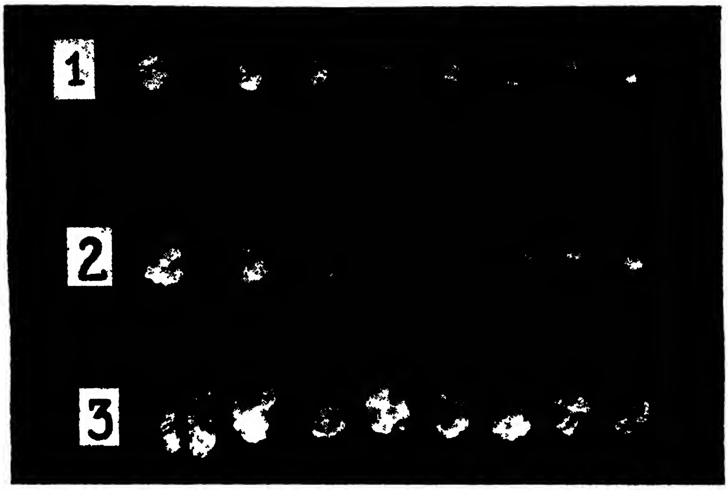
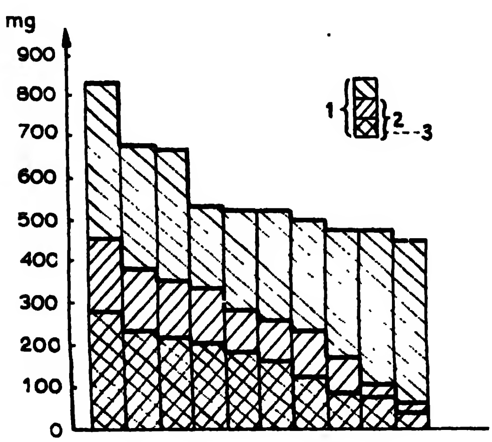
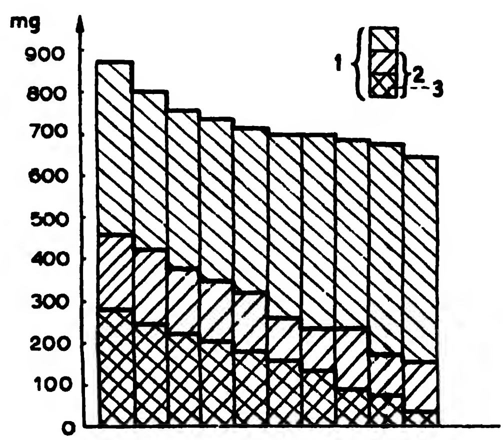


Fig. 1. Growth of carrot tissue. 1. In Nitsch medium with 2.5% glucose added. 2. The same + an extract from bean leaves grown in red light. 3. The same + an extract from bean leaves grown in blue light.

by exposure to red light (Fig. 5). Extracts from the leaves of plants which were in the dark under normal conditions (variant 4) or deprived of CO_2 (variant 5) during the final 3 h dark exposure possessed identical biological activities (Fig. 7). This establishes





Figs. 2–7. The columns designate the weight in mg of each of the 10 calluses in each variant of the experiment.

Fig. 2. 1. Extract from leaves of a bean plant grown 14 days in blue light (variant 3). 2. Extract from leaves of bean plant cultivated in red light (variant 1). 3. Control, Nitsch medium without extracts from leaves.

Fig. 3. 1. Extract from leaves of bean plant exposed 3 h in blue light (variant 10). 2. Extract from leaves of bean plant exposed 3 h in red light (variant 6). 3. Control, Nitsch medium without extract.

that it is in fact photosynthesis and not dark CO_2 fixation which is responsible for the different biological effect of the other extracts.

The chemical nature of the products of photosynthesis responsible for the effects described above cannot be deduced from the present experiments. However, from the preceding considerations it seems that these can be neither carbohydrates, auxins nor vitamins. Nitrogenous substances produced during photosynthesis may be responsible. This agrees with some previous suggestions[2] and with the fact that blue light is es-

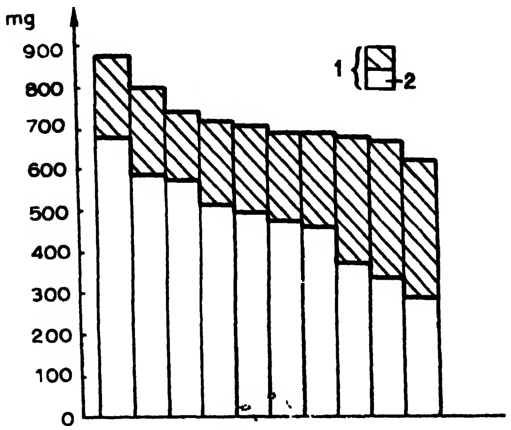


Fig. 4. 1. Extract from leaves of bean plant exposed 3 h in blue light in presence of CO_2 (variant 10). 2. The same but with CO_2 excluded (variant 11).

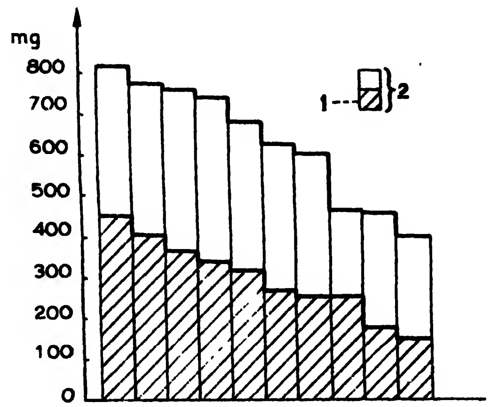


Fig. 5. 1 Extract from leaves of bean plant exposed 3 h in red light in presence of CO_2 (variant 6) 2 The same but with CO_2 excluded (variant 7).

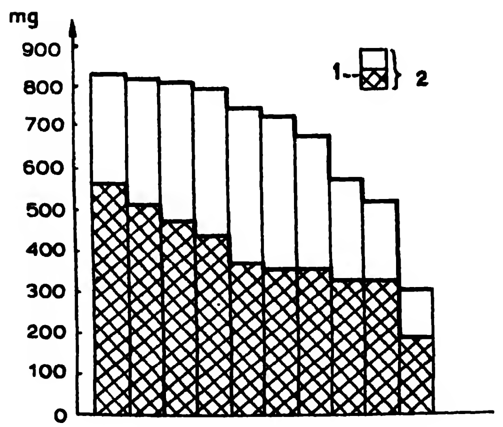


Fig. 6. 1. Extract from leaves of bean plant exposed 3 h in white light in presence of CO_2 (variant 8). 2. The same but with CO_2 excluded (variant 9).

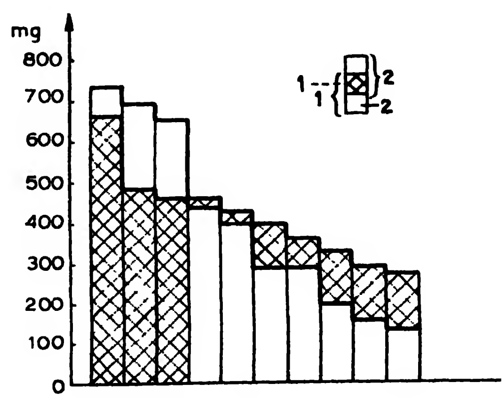


Fig. 7. Extract from leaves of bean plant kept in dark 3 h in presence of CO_2 (variant 4). 2. The same but with CO_2 excluded (variant 5).

pecially effective in the photosynthetic formation of nitrogenous substances such as amino acids and proteins[1-4]. It is also in accord with our observation that the purine and pyrimidine type of substance stimulates the growth of tissues similar to those used in this study.

References p. 178

The experiments described in the present paper thus show that during photosynthesis compounds of high physiological activity are produced whose composition and relative amounts strongly depend on the spectral composition of the light. It may be assumed that it is just these substances which give rise to the pronounced differences in the growth and development of plants cultivated in light of different spectral composition. The results also indicate that the biological test method may effectually be used in the study of the various products of photosynthesis and their physiological significance.

REFERENCES

1 A. A. Nichiporovich, *Proc. Intern. Conf. Peaceful Uses Atomic Energy, Geneva, 1955*, Vol. 12, 1955.

2 A. A. Nichiporovich, T. F. Andreyeva, N. P. Voskresenskaya, L. A. Nezgovorova and Yu. I. Novitsky, *Proc. First (Unesco) Intern. Conf., Paris, Radioisotopes in Scientific Research*, Vol. 4, London, 1958.

3 N. P. Voskresenskaya, *Fiziol. Rasteni. Akad. Nauk S.S.S.R.*, 3 (1956) 49.

4 N. P. Voskresenskaya, *Doklady Akad. Nauk S.S.S.R.*, 2 (1952) 429.

5 A. F. Kleschnin, *Die Pflanze und das Licht*, Akademie Verlag, Berlin, 1960.

6 E. C. Wassink and J. A. Stolwijk, *Ann. Rev. Plant Physiol.*, 7 (1956) 373.

7 J. A. Lockhart, *Physiol. Plantarum*, 11 (1958) 478.

8 J. P. Nitsch, *Am. J. Botany*, 43 (1956) 839.

Symposium 3

PHOTORECEPTORS IN AQUATIC ORGANISMS

Chairman: N. MILLOTT, London (Great Britain)

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FINE STRUCTURE OF AQUATIC PHOTORECEPTORS*

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Photoreceptors are structures containing photosensitive pigment-complexes (*e.g.* carotenoids chlorophyll, retinene) that upon light absorption initiate phototropisms, photosynthesis and vision. A comparative structural analysis of the plant and animal photoreceptors is in progress in our laboratory.

I would like to describe a few of the aquatic plant and animal photoreceptors' *fine structure*, ranging from the chloroplasts of algae to the vertebrate retinal rods.

PLANT PHOTORECEPTORS

The *chloroplasts* are the photoreceptors for photosynthesis; they range in size from less than 1 μ in diameter, to more than 10 μ in length. All chloroplasts, so far investigated by electron microscopy, show a lamellar structure with spacings of the order of 200 Å[1,2]. In the blue-green algae and in the photosynthetic bacteria, lamellar structures are also found; these however, are described as *chromatophores*. Pyrenoids are a part of the structure of chloroplasts of algae. They are tightly packed granular material, concerned with starch synthesis and are found within the lamellae of the chloroplast[3].

Chlorophyll *a* occurs in all plant chloroplasts, but other isomers, *b*, *c*, and *d*, are also found; in the photosynthetic bacteria there is bacteriochlorophyll. In the higher plants, the two main chlorophyll isomers are *a* and *b* in a ratio of about 3 : 1. The carotenoids also, are intimately linked with chlorophyll in the chloroplast. These are the xanthophylls, lutein and zeaxanthin, and the carotene, β-carotene. Phycoerythrin and phycocyanin are also present in the red and blue-green algae.

In *Euglena*, grown under optimal conditions of light intensity and temperature, the chloroplasts consist of regularly oriented lamellae which are dependent upon chlorophyll[1, 3]. The electron dense layers are of the order of 250 Å in thickness with less dense interspaces 300–500 Å in thickness. *Chlamydomonas*, whether grown in the light or the dark, show a complex chloroplast organization of stacks of lamellae, pyrenoid, and eyespot within the chloroplast membrane; in the absence of chlorophyll, the lamellae are not formed[4].

Although the *Euglena* chloroplasts have been studied most extensively, some observations of the photosynthetic structure in other algae are to be noted. In the blue-green algae, *Anabaena cylindrica*, there are large chromatophores that seem to be

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References p. 187/188

connected by cytoplasmic lamellae described as "chromatoplasm", whereas in *Anacytis nidulans*, only chromatophores are observable and in *Synechoccus cedrorum*, there is a cup-shaped chloroplast with bundles of lamellae. In the cryptomonads, *Cyanophora paradoxa* there are chromatophores and a distinct lamellar system, however it has been speculated that this organism has a symbiot blue-green alga; and in *Rhodomonas lens*, there are only lamellar chloroplasts and a pyrenoid, however, besides the chlorophyll and carotenoid, the organism contains a brilliant yellow fluorescent pigment (with a single peak at 585 mμ), a pteridine whose function in photosynthesis is not yet determined. The green algae include the *Euglenas* and *Chlamydomonas*, already described[1-4]. In *Volvox*, there are only lamellar chloroplasts and a pyrenoid; in the marine alga, *Valonia* (Florida and the West Indies), the chloroplasts lie embedded in the periphery of the cytoplasm in a reticular arrangement; these chloroplasts contain a pyrenoid and are also lamellar.

What does this lamellar structure tell us about the molecular structure of the chloroplast? For example, the chloroplasts of *E. gracilis* consist of 21 dense layers 250 Å in thickness. Each dense layer appears to be covered on both sides by thinner and denser layers (lamellae) 50 to 100 Å in thickness. From the geometry of the chloroplasts (diameter, length, number and total thickness of the dense layers) and the chlorophyll concentration per chloroplast, the cross-sectional area occupied by each

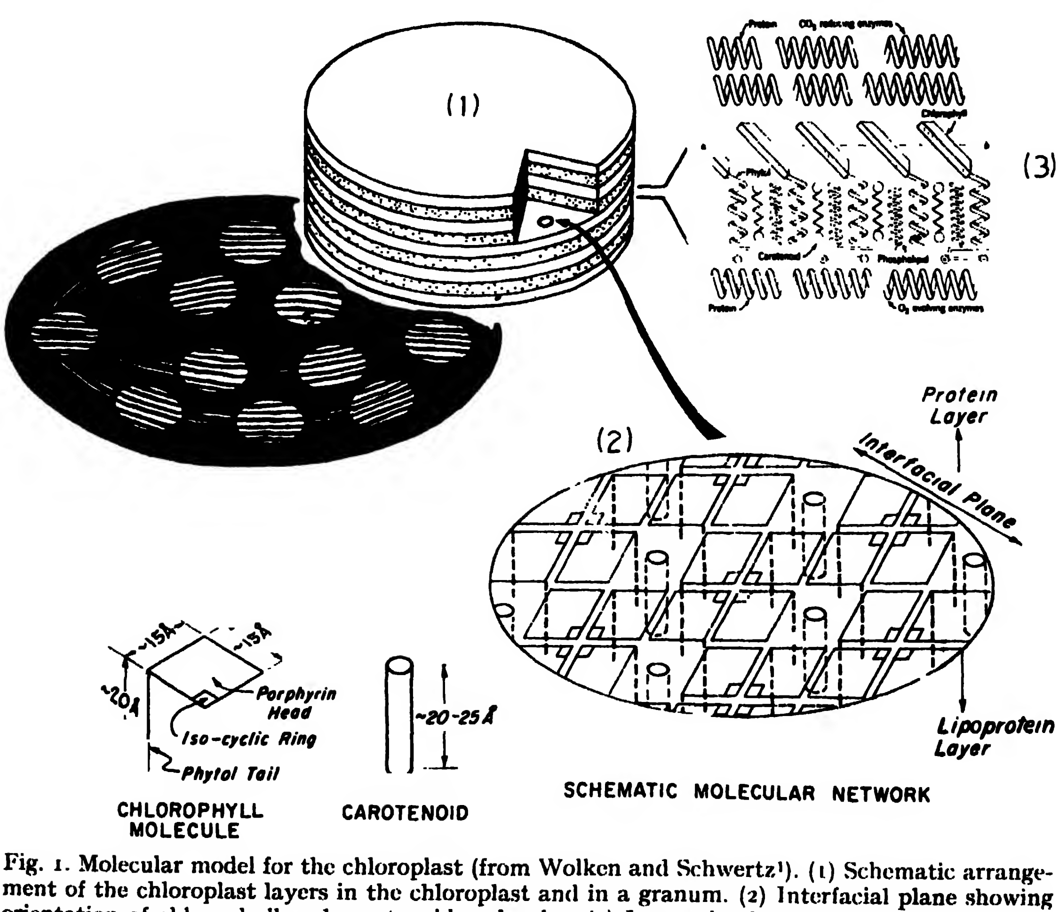

Fig. 1. Molecular model for the chloroplast (from Wolken and Schwertz[1]). (1) Schematic arrangement of the chloroplast layers in the chloroplast and in a granum. (2) Interfacial plane showing orientation of chlorophyll and carotenoid molecules. (3) Layers in the chloroplast as visualized (from Calvin[5]).

chlorophyll molecule was calculated to be 222 Å^2 in the *Euglena* chloroplast, and 246 Å^2 in the *Poteriochromonas* chloroplast[1,3].

Although the morphology and structural dimensions of the chloroplasts vary in a variety of plant species, the collected data on the chlorophyll concentration per chloroplast and geometry, indicates that the average mean area available per chlorophyll molecule in the monolayer is of the order of 200 Å^2. On the basis of these calculations, a simplified molecular model was proposed (Fig. 1). If the chlorophyll molecules are packed as a monolayer, as shown in the schematic molecular network in Fig. 1, there would still be space available at the interstitial positions between the chlorophyll molecules for the carotenoid molecules. If these spaces are occupied as illustrated, there will be one carotenoid molecule for at least every three chlorophyll molecules. This kind of close packing of the chlorophyll and carotenoid molecules in the pigment monolayers would permit energetic interaction between them. A similar model indicates that one aqueous protein layer would have CO_2-reducing enzymes and that another protein layer would have O_2-evolving enzymes[5]. If the porphin heads of the chlorophyll molecules would lie at 0° as flat plates, as indicated in Fig. 1, their greatest cross-section would be available. However, if they were oriented within the lamellae at increasing angles up to 90°, the cross-sectional area available would be decreasing. Studies of chlorophyll monolayers on various liquid surfaces, suggested that the chlorophyll molecules would probably lie at an angle near 45° within the chloroplast, then, the above calculation for the cross-sectional area of the chlorophyll molecule would be reduced to 100 Å^2. It is very likely that the absorption oscillators of these pigment molecules are arranged with an orderly orientation in a way that a maximum absorption will be observed for an incident light polarized in a certain direction.

The *eyespot* is a photoreceptor for light perception and directs the organism by phototropic reactions to light of the right wavelengths for photosynthesis. The eyespot structure has been studied in *Chlamydomonas*, *Volvox*, *Euglena*, and other organisms. In some eyespots, *lens*-like structures are found (*E. granulata*, *Volvox*). It was speculated a long time ago, that the eyespot functions analogously to the retinal cells of higher animals. Fauré-Fremiet and Rouillier[6] indicate that the second internal flagellum associated with the eyespot of *Chromulina* has a lamellar structure comparable in structure to the retinal rods. The flagellum is a sensory structure which is structurally similar to the fibril that penetrates from the outer through the inner segment of the vertebrate retinal rod[7]. Willmer[8] suggested that structurally the most interesting feature in the development of the vertebrate rods and cones is the flagellum-like fibers that connect the outer and inner segments.

In *Euglena*, the eyespot is about 2 · 3 μ and consists of the order of 50 tightly packed pigment granules (orange-red) of the order 0.1 μ in diameter that are linked with the flagella[9]. *Euglena* phototaxis and photokinesis (rate of swimming irrespective of direction) indicates that selective absorption by the eyespot is related to its photomotion[10]. The eyespot + flagellum act as a receptor-effector system, and therefore, can be considered structurally and functionally analogous to a retinal rod. Microspectrophotometric analysis of the *in vivo* eyespot of *E. gracilis*, shows a general absorption from 460–480 mμ with the major peak near 480 mμ, a secondary peak at 425 mμ, small minor peaks near 500, 530 and 590 mμ, and a broad band at 630 mμ[11]. These absorption spectra do not permit the identification of the eyespot pigments. Astaxanthin or a similar photosensitive pigment has not been isolated from green *Euglenas*.

References p. 187/188

ANIMAL PHOTORECEPTORS

Since the vertebrate eye originated in water[12] the aquatic invertebrates are of great interest. In these organisms, a variety of photoreceptor structures are found, *e.g.* eyespots, sensory cells, ocelli, and compound eyes which have evolved among annelids, molluscs, and arthropods. In the vertebrates these are retinal rods and cones. These photoreceptor structures are illustrated from a phylogenetic point of view in Fig. 2A-E. The rods have their maximal sensitivity in the blue-green at about 500 mμ; the

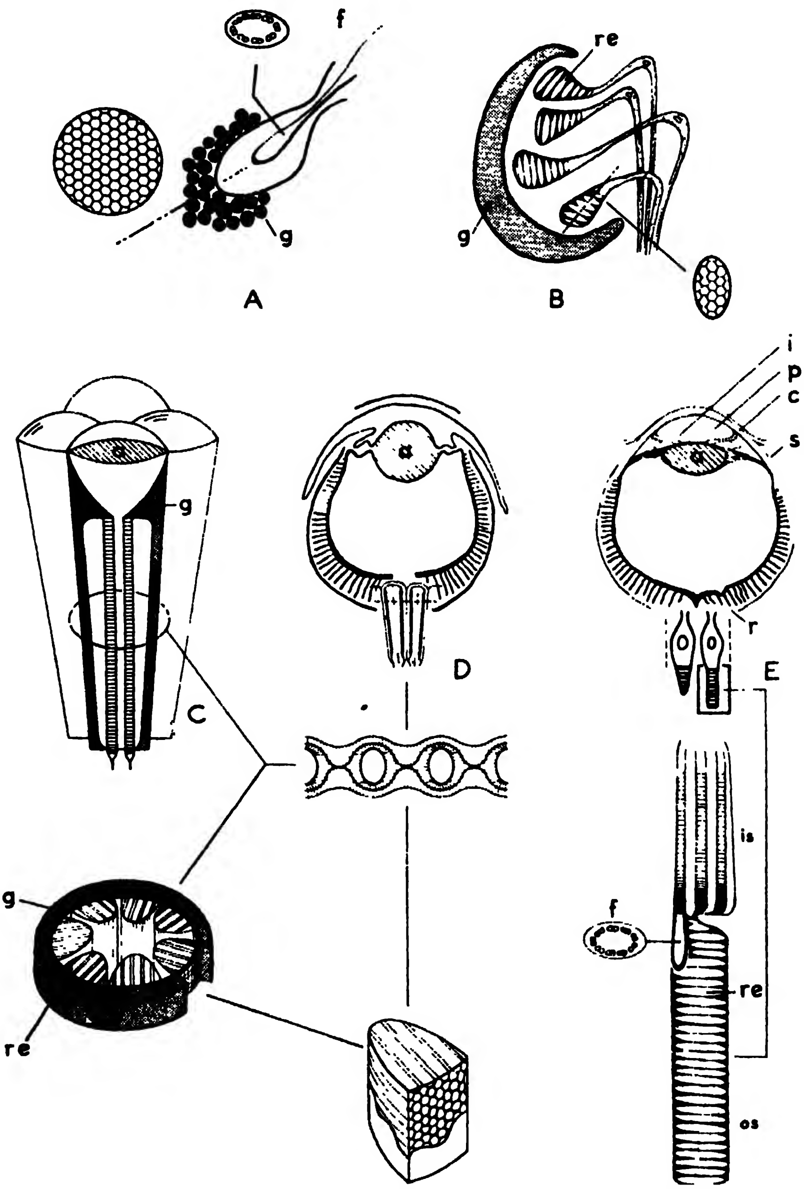

Fig. 2. Schematic phylogenetic development of the photoreceptor structures. A, eyespot and flagellum, pigment granules (g); B, sensory cells (re), surrounded by pigment granules (g); C, insect compound eye, lens (a), pigment granules (g); D, mollusc cephalopod eye showing compound retina; E, vertebrate eye, iris (i), lens (a), pupil (p), ciliary muscle (c), sclera (s) and retina (r) showing an enlarged rod (re) outer segment (os) and inner segment (is). It is to be noted the structure connecting the inner with the outer segment to that of the eyespot with flagellum of A (Modified from Wolken[2]).

cone sensitivity is transferred toward the red, lying in the yellow-green at 560 mμ. The rods and cones contain, as their photosensitive pigments, either retinene$_1$ or retinene$_2$ (the aldehydes of vitamin A_1 or A_2) linked with a protein opsin. Such extracted pigment-protein complexes, according to Wald[13], are identified by their color and absorption spectra as rhodopsin (retinene$_1$ + rod opsin) or porphyropsin (retinene$_2$ + rod opsin) for the rods; iodopsin (retinene$_1$ + cone opsin) or cyanopsin (retinene$_2$ + cone opsin) for the cones. There are from 1×10^6 to 1×10^9 rhodopsin molecules per outer segment of a retinal rod. Studies indicate that the maximum absorption peaks of these rhodopsins may be adapted to the quality of the light in the environment[14].

Sensory cells

In the flatworm, *Planaria*, the two eyes consist of pigment granules and *sensory cells*. The sensory cells' ends continue as nerves which enter the brain. The pigment granules shade the sensory cells from light in all directions but one, and so enable the animal to respond in a negative way to the direction of light. The sensory cells are structures analogous to the retinal rods of the vertebrates. These retinal structures (rods) are about 5 μ in diameter with a more variable length of approximately 35 μ and consist of lamellae that are tubules of the order of 400 Å in diameter.

The marine planarian, *Convoluta roscoffensis* (peculiar to the coast of Brittany) responds directly to light and moves from the dark areas to the light areas rapidly. They appear to have a built-in "time-clock" rising and falling with the time of the tides that is independent of light and temperature even when removed to the laboratory. However they live symbiotically with an alga (*Chlamydomonas*-like) and need light for photosynthesis. In the aquatic planarian, *Dendrocoelum lacteum*, the action spectrum has two main absorption peaks, one at 510 mμ, probably a rhodopsin, and another in the ultra-violet at 370 mμ[15].

Compound eye — retinal structure

The compound eye consists of ommatidia, each of which is made up of retinular cells; the differentiated part of the retinular cell is the rhabdomere. The rhabdomeres taken together form a rhabdome. The rhabdome is considered the "light trapping" area where the visual process is initiated.

The eyes of the insects, *Drosophila*, housefly, dragonfly, honeybee, cockroach and spider, consist of seven retinular cells radially arranged forming a cylinder[16-18]. Electron microscope studies indicate that the rhabdomeres are structurally packed tubules, which are 400 Å in diameter, whose walls are of the order 100 Å in thickness. Retinene$_1$ has been isolated from the rhabdomeres of the housefly, the honeybee and the cockroach, indicating that the insects contain a rhodopsin.

The eyes of the molluscs are provided with mechanisms for accommodation and resemble those of the vertebrate eye. The eyes and photoreceptor structures of the cephalopods, *Sepia* and *Octopus*, the gastropod, conch (*Strombus*), and *Pecten*, have been studied by electron microscopy. The photoreceptors of the retina are not inverted as in the vertebrate eye and are directly exposed to the incident light. Electron microscopy of the retina shows that the arrangement of the retinal cells is very much like the insects' ommatidia — with retinular cells and rhabdomeres. The retinas of *Sepia* and *Octopus* are made up of rhabdomes in which there are four rhabdomeres (retinal rods)

References p. 187/188

radially arranged. A central space separates the rhabdome, which contains pigment-screening granules that migrate depending on the light intensity. In both the *Octopus* and *Sepia*, each rhabdomere averages about 1 μ in diameter. Its microstructure is that of densely packed tubules (there are about 20 tubes per micron); each is about 200 Å in diameter[20-22]. The arrangement of the rhabdomeres in the king crab, *Limulus*, the crustacean, *Daphnia*, and the mollusc, *Pecten*, is similar to that of the *Octopus*. The rhabdomeres' *fine structure* is that of tubules, also with similar dimensions[19,21].

Rhodopsins have been extracted from crustacea, molluscs, and insects[23-25]. A number of these organisms also show photosensitivity to the ultra-violet, and have been shown to have color discrimination; that they may possess other photosensitive pigments cannot be excluded[26]. Many of them exhibit orientation relative to the direction of vibration of polarized light; such sensitivity to the plane of polarization suggest the existence of a polarized light analyzer within the eye.

Vertebrate — retinal rods and cones

The vertebrate animals with strictly aquatic vision include the fishes, some

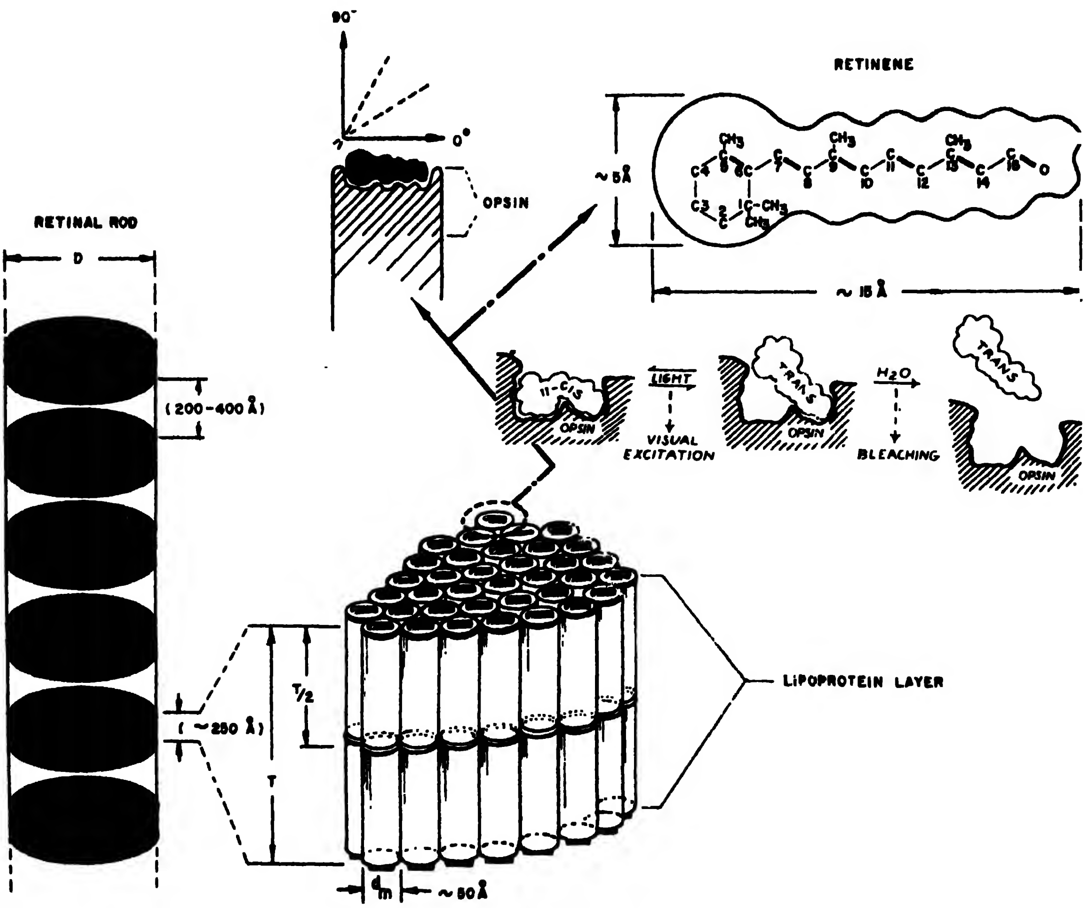

Fig. 3. Molecular model for the outer segment of the retinal rod (from J. J. Wolken, modified from *J. Cellular Comp. Physiol.*, 48 (1956) 349). The association of retinene with opsin in rhodopsin, showing the mechanism of bleaching during visual excitation (originally proposed by A. Hubbard and A. Kropf, *Ann. N. Y. Acad. Sci.*, 74 (1958) 266).

amphibians, the sea cows and the whales. In the vertebrate retinal rods and cones, the visual complex is contained only in the outer segments. The whale outer segments are from 1.0 to 1.5 μ in diameter and consist of lamellae (plates) of the order of 200 Å in thickness[22]. Other retinal rods and cones outer segments studied by electron microscopy, include the frog, chicken, guinea pig, rabbit, perch, cattle and monkey. They are all lamellae from 100–200 Å in thickness, separated by less dense interspaces 200–500 Å[22,27]. The photoreceptor geometry (length, diameter, thickness, number of dense layers and pigment concentration) was similarly used as in the chloroplast for calculating the cross-sectional area of the pigment macromolecule, rhodopsin. For example, for cattle and frog rhodopsin, the cross-sectional area is 2500 $Å^2$; the diameter of the molecule would then be of the order of 50 Å, which is about the right order of magnitude for the rhodopsin molecule[28]. A molecular model for the retinal rod outer segment, based on the geometry from electron microscopy, pigment concentration, and the above calculation, was proposed (Fig. 3).

Retinal pigment globules

In the retina of certain animals, there are colored oil globules which are believed to have evolved a long way back, since they are found in the retinas of fish as ancient as *Chondrosteans* and sturgeons. Modern fish have on the other hand, discarded them, but lungfish still have them. It is implied that they are involved in color vision and have always been a characteristic of the vertebrate cone[29]. The relative ease with which the colored globules may be identified *in situ* in the retina, and their absorption spectra determined with the microspectrophotometer, makes possible their study[30]. What function they may have in the visual process is still unknown, but they have long been suspected of acting as color filters for the retinal cones. Since a red globule, for instance, absorbes light somewhere in the blue-green region, the location of the globule between the inner cone segment and the light source, makes a color filter theory reasonable. However, there is no direct correlation between the color of the globule and the color discrimination of animals possessing them.

REFERENCES

1 J. J. Wolken and F. A. Schwertz, *J. Gen. Physiol.*, 37 (1953) 111.
2 J. J. Wolken, *Ann. Rev. Plant Physiol.*, 10 (1959) 71.
3 J. J. Wolken and G. E. Palade, *Ann. N.Y. Acad. Sci.*, 56 (1953) 873.
4 R. Sager and G. E. Palade, *J. Biophys. Biochem. Cytol.*, 3 (1957) 463.
5 M. Calvin, Brookhaven Symposia in Biology, No. 11, *The Photochemical Apparatus: Its Structure and Function*, 1959, p. 160.
6 E. Fauré-Fremiet and C. H. Rouiller, *Exptl. Cell Research*, 14 (1958) 47.
7 E. De Robertis, *J. Biophys. Biochem. Cytol.*, 2 (1956) 319.
8 E. N. Willmer, *Ann. Rev. Physiol.*, 17 (1955) 339.
9 J. J. Wolken, *J. Protozool.*, 3 (1956) 211.
10 J. J. Wolken and E. Shin, *J. Protozool.*, 5 (1958) 39.
11 G. K. Strother and J. J. Wolken, *Nature*, 188 (1960) 601.
12 G. L. Walls, *The Vertebrate Eye*, Cranbrook Institute of Science Press, Bloomfield Hills, Mich., 1942.
13 G. Wald, *Handbook of Physiology - Neurophysiology I*, American Physiological Society Publishers, Washington, 1959, p. 671.
14 F. Crescitelli, *Ann. Rev. Physiol.*, 22 (1960) 525.
15 M. H. Pirenne and F. H. C. Marriot, *Nature*, 175 (1955) 642.
16 J. J. Wolken, J. Capenos and A. M. Turano, *J. Biophys. Biochem. Cytol.*, 3 (1957) 441.
17 H. Fernández-Morán, *Exptl Cell Research*. Suppl. 5 (1958) 586.

[18] T. H. GOLDSMITH AND D. E. PHILPOTT, *J. Biophys. Biochem. Cytol.*, 3 (1957) 429.
[19] W. H. MILLER, *J. Biophys. Biochem. Cytol.*, 3 (1957) 421.
[20] J. J. WOLKEN, *Trans. N.Y. Acad. Sci.*, Ser. II, 19 (1957) 315.
[21] J. J. WOLKEN, *J. Biophys. Biochem. Cytol.*, 4 (1958) 835.
[22] J. J. WOLKEN, *Ann. N.Y. Acad. Sci.*, 74 (1958) 164.
[23] R. HUBBARD AND G. WALD, *Nature*, 186 (1960) 212.
[24] P. K. BROWN AND P. S. BROWN, *Nature*, 182 (1958) 1288.
[25] T. GOLDSMITH, *Ann. N.Y. Acad. Sci.*, 74 (1958) 223.
[26] J. M. BOWNESS AND J. J. WOLKEN, *J. Gen. Physiol.*, 42 (1959) 779.
[27] F. S. SJÖSTRAND, *J. Cellular Comp. Physiol.*, 42 (1953) 15, 45.
[28] G. WALD, *Science*, 119 (1954) 887.
[29] J. H. PRINCE, *Comparative Anatomy of the Eye*, Charles H. Thomas, Springfield, Ill., 1956, p. 65.
[30] G. K. STROTHER AND J. J. WOLKEN, *Exptl. Cell Research*, 21 (1960) 504.

PHOTORECEPTION OF A PLANKTONIC CRUSTACEAN IN RELATION TO LIGHT PENETRATION IN THE SEA

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For several years we have been concerned with the ecology of the euphausiid crustaceans and, in particular, with their photobiology. This paper summarizes our findings and presents some of our thoughts on the subject at this time.

We need not elaborate again the arguments for using the migratory sonic-scattering layer in the coastal waters off San Diego as an indication of the depth at which euphausiids may be found. The works of Boden[1] and Tucker[12], as well as our collections from 1954 to 1960, have shown that when a scattering layer can be detected in the area, euphausiids will be in it, and that when the layer splits at sunset, euphausiids are present in the upper part of the layer.

The depth of this migratory scattering layer off San Diego is associated with the depth of an isolume (about $1 \cdot 10^{-4}$ μWatts/cm^2) as detected by a 931-A multiplier phototube (Kampa and Boden[2]). This association is especially close during the rapid migration of the layer at twilight.

In 1955, we first used our present bathyphotometers (Boden, Kampa and Snodgrass[3]), and the response of these is rapid enough that we have been able to show that the light within the scattering layer is modified by the luminescence of the animals themselves (Kampa and Boden[4]; Boden and Kampa[5]). The luminescence is greatest during the vertical migrations of the animals at twilight when their association with the constant level of transmitted sun- and sky-light is most marked. The luminescence appears on the bathyphotometer record as a mass of flashes, often of such frequency and intensity as to cause an apparent increase of ambient light with depth. The animal light is blue-green in color with an energy spectrum that peaks near 478 mμ.

In the laboratory we have measured the luminescence of three euphausiid species from separate genera: *Euphausia pacifica* (Kampa and Boden[4]), *Thysanoëssa raschii* (Boden and Kampa[6]), and *Meganyctiphanes norvegica* (unpublished results). The emission curve is bimodal in each species, with a sharp primary peak near 476 mμ and a second lower peak between 520 and 540 mμ. The intensity of the glow from an individual is about 20 times the intensity of transmitted skylight with which the scattering layer is associated.

A photosensitive pigment, which we have called euphausiopsin, has been extracted in aqueous solution from dark-adapted eyes of euphausiids from Californian coastal waters (Kampa[7]), and its presence in *Meganyctiphanes norvegica* has been reported by Dartnall (1957, personal communication) and Fisher and Goldie[8]. The pigment

References p. 196

absorbs light maximally between 460 and 465 mμ, and it is bleached by light to release first retinene (Fisher and Goldie[8]) and then vitamin A (Kampa[7]).

The new material which we should like to present here is twofold. At sea, we have examined the spectral characteristics of the light in the water column above the sonic-scattering layer and the changes in this spectrum during the twilight migrations of the layer. In the laboratory, we have begun electrophysiological measurements of the sensitivity of the euphausiid eye.

PHYSICAL MEASUREMENTS AT SEA

The photometers used were of the type described by Boden, Kampa and Snodgrass[3]. Since these employ multiplier phototubes and a series of interference-type filters with peaks at intervals between 410 and 634 mμ, they permit measurement of near-monochromatic light of low intensity. An automatic filter changer places a succession of 5 filters in the light path. The window of the photometer is opal plastic with the characteristics of a Lambert cosine collector. A collimator restricts the angle of incidence on the photocathode to the useful 5° cone of the interference filters. The instrument measures irradiance.

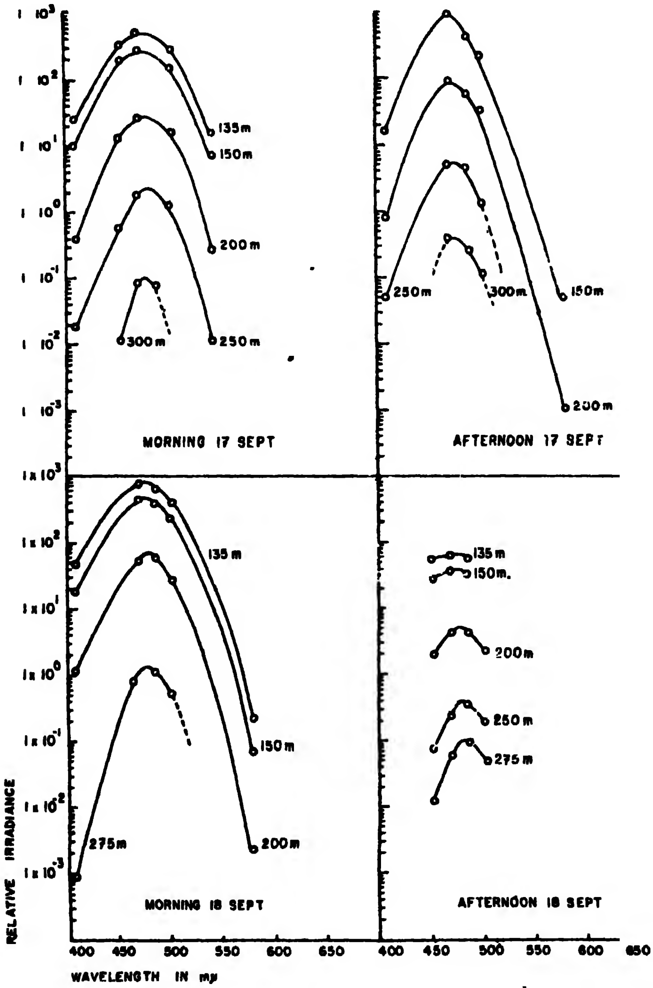

Fig. 1. Relative irradiance spectra at indicated depths off San Diego in September.

The water column above the sonic-scattering layer

The first series of measurements was made to determine midday conditions in the water column down to the depth of the scattering layer. The photometer was oriented to look upward. It was lowered immediately before and after noon on two consecutive days in September.

The light at depths between 135 and 300 m was blue-green and peaked between 475 and 480 mμ (Fig. 1) whichever combination of filters was used. The peaks of the curves obtained become increasingly sharp with depth as the sea water acts as a monochromator (Tyler[9]).

Values of the extinction coefficient (k) for each wavelength at different depths are shown in Table I. The extinction coefficient at depth z is defined by

$$k_\lambda = \ln H_{\lambda,z} - \ln H_{\lambda,(z+1)}$$

where $H_{\lambda,z}$ and $H_{\lambda,(z+1)}$ represent irradiance values of wavelength λ on horizontal surfaces at depths z and (z + 1 m).

TABLE I

EXTINCTION COEFFICIENTS (k) OF IRRADIANCE AT WAVELENGTHS (λ) AT DEPTHS (D)

D	λ	k
150–250	410	0.065
	452	0.053
	470	0.051
	489	0.048
	503	0.047
	541	0.064
122–198	581	0.081

The extinction coefficient is defined as $k_\lambda = \ln H_{\lambda,z} - \ln H_{\lambda,(z+1)}$ where z and (z + 1) represent 1-m intervals of depth (D).

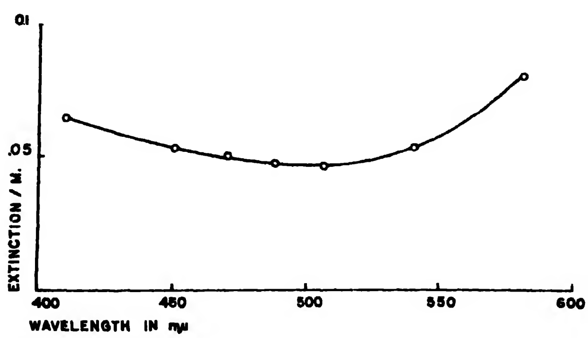

Fig. 2. Extinction coefficients at various wavelengths at depths between 150 and 250 m off San Diego in September.

The values presented were obtained in the water mass below the photic zone and above the scattering layer, where k was constant and the attenuation curves were fairly straight, which suggests that there was little interference from biological or geological sources.

Fig. 2 shows extinction per meter for various wavelengths, and it can be seen that

the water was rather clear for shelf water. According to the data of Utterback[10] and Jorgensen and Utterback[11], it would be typified as average oceanic water.

Rate of attenuation at the surface and at depth at sunset

To determine the difference in the rate of attenuation at two different depths two identical photometers were used. One was suspended 10 m below the surface from a rubber float, the other was suspended 200 m directly below. No filter was used in either. Separate recorders were used, and simultaneous recordings were made.

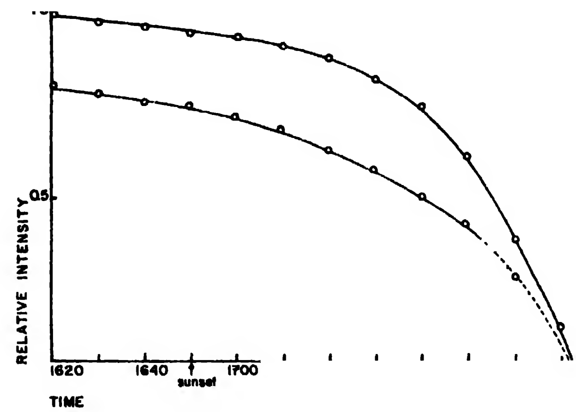

Fig. 3. Simultaneous rates of light decay at 10 m (upper curve) and 200 m (lower curve) during sunset off San Diego in September. The broken line indicates the onset of bioluminescence

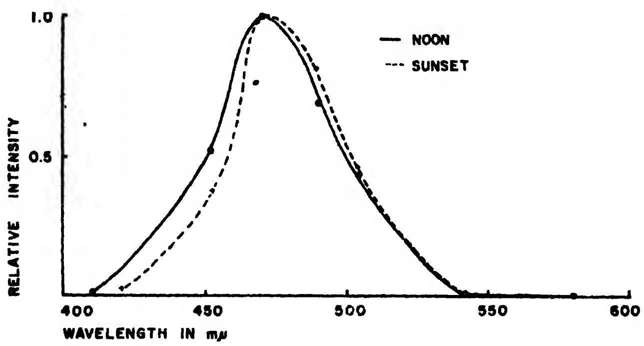

Fig. 4. Spectral distribution of transmitted sun- and sky-light at 170 m at noon and sunset off San Diego in September.

Fig. 3 shows that the rate of extinction was very similar at the two depths from 30 min before sunset until 50 min after sunset. From that time on the curves approach each other, with the rate of extinction at the surface being apparently more rapid than at 200 m. We suggest that this deeper picture is obscured by bioluminescence.

A strong scattering layer was present on this evening, and 50 min after sunset this layer was shoaler than 200 m. Shortly after 1750 h the bioluminescence at 200 m was so marked and the flashes so large that it was not possible to obtain sky-light readings at that depth.

Spectral distribution of light at a constant depth of 170 m at sunset

The photometer was lowered on three successive days, at noon and sunset, to a predetermined depth of 170 m. The values in Fig. 4 represent the average of the values obtained on each of the three days.

It can be seen that there is a strong shift to the longer wavelengths at sunset. The light being measured was, of course, a combination of sun- and sky-light, and this shift is possibly due to selective refraction at the sea surface at a critical point during sunset.

Spectral composition of transmitted sun- and sky-light just above the sonic-scattering layer during its twilight migration

The photometer was equipped with the automatic filter changer, and a variety of filters was used. The instrument was lowered to just above the sonic-scattering layer, and the changer was allowed to rotate. The intensity observed at 470 mμ was selected as a reference value. During light decay at sunset the photometer was raised to follow this 470-mμ isolume.

The ship was far from shore, and ship lights were doused. Only sky-light or possibly bioluminescence, although it was not recognizable as such, was bei..g recorded.

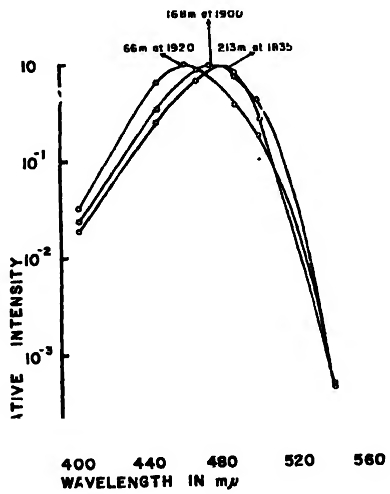

Fig. 5. Spectral composition of light just above the scattering layer during it twilight migration toward the surface off San Diego in September.

Fig. 5 shows the average values obtained at similar depths, similar times and similar wavelengths during three successive twilight periods. It can be seen that the peak values shift from about 490 mμ at sunset toward shorter wavelengths to about 465 mμ at the end of twilight.

ELECTROPHYSIOLOGICAL EXPERIMENTS IN THE LABORATORY

Rather than attempt to design valid laboratory behavioral studies for animals that may well never encounter an interface in their natural lives, we have depended on biochemical and electrophysiological experiments to determine the visual sensitivity of the euphausiids.

References p. 196

Electroretinograms have been obtained from the eyes of a number of species of euphausiids. A tungsten grid serves as the grounded neutral electrode, and the living animal rests on this in a shallow bath of sea water at 7–10°C. A tungsten microelectrode (diameter 2–3 μ), insulated to near the tip is inserted into the eye. The signal from the recording electrode is transmitted via a Grass P-6 preamplifier (with probe) to a Tektronix oscilloscope with a Polaroid Land camera.

The stimulating light is a uniform field, and flash duration is determined by a diaphragm shutter which also triggers the oscilloscope. Interference filters are used to select various wavelengths in color experiments, and neutral density filters modify intensity. Light intensities at the level of the euphausiid eye are determined directly with a thermopile and galvanometer calibrated against a U.S. Bureau of Standards source.

The structure of the euphausiid eye differs greatly from genus to genus. So far, we have worked with two species having spherical eyes (*Euphausia pacifica* and *Meganyctiphanes norvegica*) and two with bilobed eyes (*Thysanoëssa gregaria* and *Nematoscelis difficilis*).

Responses from the upper and lower lobes of bilobed eyes are quite distinct. The main wave of the electroretinogram of the upper lobe is a fast response, lasting 20–30 msec. Its duration and amplitude are independent of the duration of the stimulus. The main wave of the ERG in the lower lobe is a slow response which varies in duration, but not in amplitude (through the range of shutter speeds we have used), with the duration of the stimulating flash.

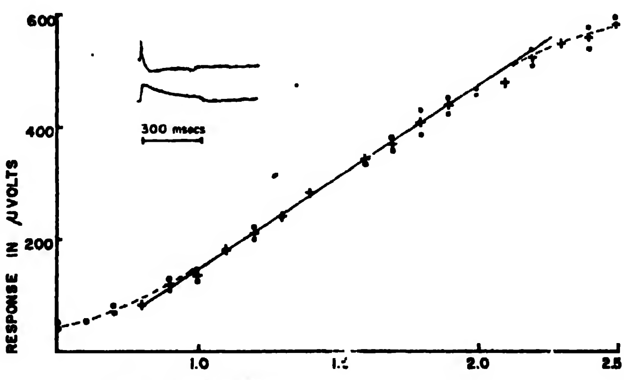

Fig. 6. Relationship of intensity of stimulus to degree of response in the eye of *Euphausia pacifica*. Inset: Upper, the fast, and Lower, the slow response of *E. pacifica* to a 300 msec flash.

Both fast and slow components are present in the spherical eye of *E. pacifica* (Fig. 6). The fast response, like that recorded from the upper lobe of a bilobed eye, is independent of flash duration, whereas the duration of the slow response varies directly with the duration of the stimulus. In some experiments with *E. pacifica* only the slow response has been detected, possibly because of differences in microelectrode placement.

The main wave of the ERG may be either a positive or a negative deflection in any one experiment. This is probably due to differences in depth of penetration by the microelectrode.

The amplitude of both fast and slow responses varies directly with the logarithm of the stimulating intensity through a range of 1 to 2 orders of magnitude (Fig. 6). The slope of the log I-response curve varies from animal to animal, but in any one experiment the linear relationship is consistent.

Our interest in the colors of the lights encountered and produced by euphausiids has led us naturally to studies of the relative sensitivity of their eyes at different wavelengths. Since the relationship between the logarithm of the stimulating intensity and the amplitude of response is linear, spectral sensitivity may be measured either be exposing the eye to restricted wave bands of an equal energy spectrum or by varying the intensity of the stimulus to produce an equal biological response at each wave band. Both approaches have been used, first on the spherical eyes of *E. pacifica* and *M. norvegica*, and more recently on the bilobed eye of *N. difficilis*. The results with spherical eyes were so confusing as to seem meaningless until the bilobed eye was examined.

The curves representing spectral sensitivity of the lower and upper lobes of *N. difficilis* are quite different. The lower lobe (Fig. 7, A) is almost uniformly sensitive to a wide band from 460–515 mμ, with a sharp peak near 490 mμ. The upper lobe (Fig. 7, B) is most sensitive between 460 and 470 mμ, with a secondary peak near 530 mμ. The response curve of the upper lobe is interesting, for its primary peak is in good accord with the difference spectrum of the photosensitive pigment euphausiopsin (Fig. 7, C). This is the first evidence that the pigment participates in the vision of euphausiids.

Fig. 7. Spectral sensitivities of: A, *N. difficilis* lower lobe; and B, *N. difficilis* upper lobe; C, Difference spectrum of euphausiopsin; D. *M. norvegica*; E, *E. pacifica*; F, Artificial curve obtained by adding spectral sensitivities of upper and lower lobes of *N. difficilis* at each wavelength. Values representing sensitivity and difference spectrum have been equated to 1 at 460 mμ and the two families of curves have been separated for clarity of presentation.

References p. 196

The spectral sensitivity curves of spherical eyes are unlike either of those from the bilobed eye. *M. norvegica* (Fig. 7, D) is about equally sensitive to lights of 460 and 515 mμ, with a third, lower peak of sensitivity near 490 mμ. *E. pacifica* (Fig. 7, E) is most sensitive to light near 460 mμ, with a second peak near 490 mμ.

When the responses of the upper and lower lobes are added at each wavelength, an artificial curve representing the total spectral sensitivity of *N. difficilis* results (Fig. 7, F). This curve is similar to the response curve of *E. pacifica*.

Either a peak or plateau is apparent near 460 mμ in each of the response curves, and euphausiopsin may be responsible for the sensitivity of all three species to the 420–480-mμ region of the spectrum. Euphausiopsin cannot account for the peaks of sensitivity near 490 and 515 mμ, however. We suggest that euphausiopsin and two additional light-sensitive substances may function in the vision of euphausiids, and that the ratios of their concentrations vary from species to species and from place to place in an individual eye.

ECOLOGICAL CONSIDERATIONS

Adult euphausiids of the Pacific species discussed here are intimately adapted to their photic environment, and this has helped solve certain of the problems confronting them. They are large, succulent animals, eagerly sought as food, and they must avoid predators. They achieve this, in part at least, by living at a light level so low that they must be invisible most of the time. During the day this light level is at a depth which is barren except for detrital fall-out. The complicated visual system of the euphausiids must permit them to detect very slight changes in light intensity, and they follow the isolume to which they are attuned to the phytoplankton-rich surface layers where they may feast at night.

Euphausiids must also remain in a community to avoid specific suicide. Here again, light sensitivity seems important, and luminescent flashing and the ability to detect and recognize such flashing may well serve to keep together like animals living at a light level too low to permit ordinary recognition of form.

Recognition of the relationship of the wavelengths between 460 and 490 mμ in the physical environment of these animals to their visual mechanisms — the fast response associated with 460 mμ, and the slow response associated with 490 mμ — and to their luminescence midway between these wavelengths is too recent to permit anything but speculation on its significance at this time.

REFERENCES

1 B. P. BODEN, *USNEL Rept.*, 186 (1950) 1.
2 E. M. KAMPA AND B. P. BODEN, *Nature*, 174 (1954) 869.
3 B. P. BODEN, E. M. KAMPA AND J. M. SNODGRASS, *J. Marine Biol. Assoc. United Kingdom*, 39 [2] (1960) 227.
4 E. M. KAMPA AND B. P. BODEN, *Deep-Sea Research*, 4 (1957) 73.
5 B. P. BODEN AND E. M. KAMPA, *Pacific Sci.*, 11 (1957) 229.
6 B. P. BODEN AND E. M. KAMPA, *Nature*, 184 (1959) 1321.
7 E. M. KAMPA, *Nature*, 175 (1955) 996.
8 L. R. FISHER AND E. H. GOLDIE, *Proc. XVth Intern. Cong. Zool.*, 1958, p. 533.
9 J. E. TYLER, *Limnol. Oceanog.*, 4 (1) (1959) 102.
10 C. L. UTTERBACK, *Conseil Perm. Intern. p. l'Explor. de la Mer, Rapp. et Proc.-Verb.*, 101 [2] No. 4 (1936) 15 pp.
11 W. JORGENSEN AND C. L. UTTERBACK, *J. Marine Research (Sears Foundation)*, 2 (1939) 30.
12 G. H. TUCKER, *J. Marine Research (Sears Foundation)*, 10 [2] (1951) 215.

LE SENS DERMATOPTIQUE, MODE PRIMITIF DE PHOTORÉCEPTION, ENVISAGÉ SPÉCIALEMENT CHEZ LES ORGANISMES AQUATIQUES

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HISTOIRE DE LA NOTION DE SENS DERMATOPTIQUE

Le sens dermatoptique paraît avoir été découvert par Pouchet[1], en 1872: cet auteur observa que des larves de Mouches (*Lucilia caesar, Eristalis tenax*), qui sont dépourvues d'yeux, sont néanmoins sensibles à la lumière et manifestent ce que J. Loeb appela par la suite du phototropisme négatif.

En 1884, Graber[2] écrivit un mémoire d'ensemble dans lequel il relate ses expériences concernant l'existence d'un sens dermatoptique ("Helligkeits- und Hautfarbensinn") chez des espèces très diverses: des Lombrics, des Blattes aveuglées, des Tritons aveuglés; ses conclusions sont certainement entachées d'erreur, mais il eut le mérite de monter le premier que la sensibilité spectrale du sens dermatoptique est très différente de celle des formations oculaires en général: le sens dermatoptique est excité au maximum par les radiations visibles dont les longueurs d'onde sont les plus courtes. En 1886, Plateau[3] signale l'existence d'une sensibilité dermatoptique chez des Myriapodes aveugles du genre *Cryptos*; dans ses expériences il élimina soigneusement l'influence possible de la température et de l'humidité. En 1890 et en 1892 paraissent deux mémoires de Raphaël Dubois[4,5], l'un sur le Protée aveugle des grottes de Carniole, l'autre sur la Pholade dactyle: le Protée, photonégatif, est surtout sensible au bleu, mais la Pholade a une sensibilité spectrale qui se rapproche beaucoup de la sensibilité visuelle (maximum vers 550 mμ). Dès 1891, Willem[6] écrit un résumé historique et critique sur les "perceptions dermatoptiques" et conclut qu'on a constaté l'existence de telles perceptions chez des animaux appartenant à presque tous les groupes zoologiques, au moins chez toutes les formes à tégument mince; il fait de justes réserves sur le caractère "chromatique" de ces perceptions, tenu pour évident par Graber[2]. En 1894, Nagel[7], étudiant les sens primitifs des animaux inférieurs, met en évidence la photosensibilité d'un grand nombre de formes marines: Hydraires, Vers tubicoles, Mollusques gastéropodes et bivalves, etc. . . Il adopte l'hypothèse de Wundt[8] (1874) sur l'existence d'un "sensorium cutané commun", à la fois sensible aux excitations mécaniques, chimiques, thermiques et "photoskioptiques" et d'où seraient dérivés les sens spécialisés: sens tactile, osmique, thermique, visuel. Puis l'attention paraît se détourner, pendant un bon nombre d'années, de la question de la photoréception cutanée.

Après quelques recherches expérimentales précises de Laurens[9] (1911), établissant nettement l'existence d'un sens dermatoptique chez les têtards de deux Crapauds américains, *Bufo americanus* et *B. fowleri*, et traçant la courbe de sa sensibilité spec-

Bibliographie p. 202

trale (croissance continue de l'orangé au violet), il faut attendre l'année 1921 pour voir reparaître ces problèmes, avec les travaux de Manquat[10]. Celui-ci, ayant verni les ocelles des chenilles de *Leucoma phaeorrhaea*, observa que leur phototropisme n'est pas affecté par cette opération. En 1921 aussi paraissent les observations de Forel[11]: ayant repris les expériences de Lubbock qui établissent la grande sensibilité des Fourmis à l'ultra-violet, il conclut que ces Insectes perçoivent ces radiations principalement par leurs yeux et que leurs sensations "photodermatiques" sont plus faibles que celles des animaux inférieurs étudiés par Graber[2]. En 1925, Lammert[12] montre que des chenilles de diverses espèces ont des ocelles et un sens dermatoptique qui les fait se diriger vers la lumière.

En 1929, Hesse[13] met au point la question des photorécepteurs primitifs dans un article du "Handbuch der Physiologie" de von Bethe. Il déclare que "ce serait trop s'avancer que de conclure à une fonction dermatoptique partout où il y a réaction à la lumière chez des animaux chez lesquels on ne connaît pas de récepteurs différenciés". Et il donne en exemple le Ver de terre qui présente, éparses dans ses téguments, des cellules spéciales qui paraissent bien être des photorécepteurs rudimentaires.

Pour Pieron[14] (1937) la sensibilité dermatoptique est généralement présente sur toute l'étendue du tégument des Invertébrés à corps nu, mais il peut y avoir dans certains cas des cellules réceptrices spécialisées, chargées de pigments sensibilisateurs; en outre, chez des animaux marins très transparents, des parties diverses du système nerveux peuvent également être photosensibles, etc..

En 1938, et jusqu'en 1950, Viaud[15–23] publie une série de travaux et de mémoires sur le phototropisme animal et sa liaison avec le sens dermatoptique chez divers animaux aquatiques (Cladocères, Rotifères, Planaires). En voici les conclusions essentielles:

(*1°*) L'attraction phototropique, chez ces animaux inférieurs, est le fait du sens dermatoptique; les yeux, quand il y en a, n'assurent que l'orientation axiale de l'animal dans la lumière.

(*2°*) Le sens dermatoptique est une fonction primitive de cellules ou de tissus non différenciés quant à la photoréception, donc non chargés de pigments photosensibles spéciaux (dérivés caroténiques). Sa courbe de sensibilité spectrale est, par suite, totalement différente de celle des formations photoréceptives spécialisées et des yeux; elle croît de manière continue en raison inverse de la longueur d'onde de lumières monochromatiques, du rouge au violet.

(*3°*) Le sens dermatoptique ne donne pas lieu à des réactions perceptives, mais au phototropisme et à des réactions photophobiques ou photopathiques.

Bref, nous avons restreint la notion de sens dermatoptique de Graber[2], en l'appliquant à la seule photoréception du protoplasme banal, à l'exclusion des récepteurs tégumentaires spécialisés. Mais, par ailleurs, nous avons élargi cette notion en l'étendant à l'absorption de la lumière par tous les tissus, cutanés ou non, à condition qu'ils possèdent des propriétés photochimiques semblables à celles des systèmes photorécepteurs principalement en jeu dans l'attraction phototropique des animaux inférieurs aquatiques.

EXAMEN CRITIQUE DE LA NOTION DE SENS DERMATOPTIQUE

Il est certain que la position que nous avons adoptée soulève bien des problèmes: (*1°*) S'agit-il, comme le terme ***dermatoptique*** semble l'indiquer et comme le pen-

saient Graber[2] et Willem[6], d'une sorte de vision primitive, impliquant la mise en oeuvre d'une téléréception photique et par conséquent une projection spatiale des impressions lumineuses en un ensemble comparable à un champ visuel?

Le terme de sens *photodermique* (Hautlichtsinn) semble à vrai dire mieux approprié, car il désigne simplement une sensibilité de contact d'un type spécial permettant la perception de plages lumineuses directement projetées sur le tégument; l'orientation phototropique d'animaux sans yeux s'expliquant, à la rigueur, par la perception d'un gradient de luminosité à la surface du corps, sans qu'il soit besoin d'invoquer une analyse spatiale du champ lumineux.

(2°) Sommes-nous bien fondé à exclure du sens photodermique les organes différenciés, disséminés dans les tissus? De tels organes ont été mis en évidence notamment chez le Lombric (Hesse[13],) chez la Mye des sables et chez divers Echinodermes (Millott[24]).

La distinction du sens dermatoptique *stricto sensu* et de la sensibilité des photorécepteurs rudimentaires épars dans le tégument ne se justifie que si nous pouvons montrer qu'elle déborde le cadre de la morphologie et qu'elle se retrouve notamment au niveau physiologique des mécanismes de l'excitation photique et au niveau psychologique de la commande des comportements. Dans la revue qui va suivre, les faits expérimentaux seront donc examinés à ces trois points de vue différents.

EXPOSÉ DÉTAILLÉ DES FAITS ET ÉTAT ACTUEL DE LA QUESTION

Si l'on réserve le cas de quelques larves d'Insectes, il semble que le sens dermatoptique n'existe vraiment que chez les Animaux aquatiques ou amphibies, jusqu'aux Batraciens inclus: chez les Amibes (Mast[25]); chez des Coelentérés sans ocelles, comme l'Hydre (Trembley[26]); des Vers non-oculés, par exemple la Planaire *Castrada* (Viaud[22]); des Rotifères sans yeux, comme *Asplanchna brightwelli* (Viaud[19]), chez l'Oursin *Diadema* (Millott[24]); chez des Mollusques Gastéropodes comme *Aplysia limacina* (Dijkgraaf[27]); chez des Cladocères aux yeux extirpés (expériences de Schulz[28], sur *Daphnia pulex*, de Harris et Mason[29] sur *D. magna*, 1956); chez un Amphipode hypogé sans yeux, *Niphargus orcinus virei*, tout récemment étudié par Ginet[30]; enfin chez des Batraciens, comme le Protée aveugle (Dubois[4]) et les Crapauds du genre *Bufo* étudiés par Laurens[9]*.

Le cas des Poissons doit être examiné à part: aveuglé expérimentalement, le Vairon *Phoxinus laevis* réagit à la lumière par des changements de coloration, non à la suite de l'éclairement d'une région quelconque de la peau, mais de l'aire pinéale (Von Frisch[33]) ou d'autres régions du diencéphale (Scharrer[34]). Par contre les Poissons cavernicoles aveugles semblent bien dotés d'un sens dermatoptique (voir la revue de la question faite par Thines[35], à propos de *Caecobarbus geertsii*), mais il ne semble pas que les radiations monochromatiques aient, sur cette sensibilité, une influence variable selon leur longueur d'onde.

* Une comparaison avec les résultats des botanistes (*cf.* Haig[31]; Bünning[32]) montre qu'il y a chez les Plantes des dispositifs photorécepteurs comparables à ceux que l'on rencontre chez les Animaux et qui jouent un rôle essentiel dans le phototropisme: la pointe des coléoptiles d'Avoine est chargée d'un pigment caroténique ("oeil de carotène", sensible au maximum aux radiations de 475-500 mμ de longueur d'onde), tandis que le protoplasme "incolore" de la tige a une sensibilité spectrale qui croît de l'orangé au violet extrême. L'oeil de carotène est beaucoup plus sensible que le protoplasme banal. Mais celui-ci suffit pourtant à induire des courbures phototropiques.

Bibliographie p. 202

Chez de nombreux animaux aquatiques oculés, il est possible de mettre en évidence, dans des expériences portant sur le phototropisme, un sens dermatoptique ou une sensibilité du même type, existant au niveau de différents organes. Ainsi, chez les Daphnies, nous avons montré[16], en dehors de la sensibilité oculaire, une sensibilité photique du corps de l'animal, caractérisée par un maximum réactionnel en lumière violette; ce fait a été confirmé, en 1958, par Scheffer, Robert et Medioni[36] qui ont établi, en outre, une liaison entre l'éclairement de diverses parties du corps de la Daphnie et les mouvements de rotation de l'oeil composé. Chez les Planaires (*Planaria lugubris, P. gonocephala, P. alpina, Dendrocoelum lacteum*) la grande sensibilité aux rayons violets et ultra-violets était connue depuis longtemps; et le sens visuel et le sens dermatoptique avaient été distingués par d'assez nombreux auteurs (Parker et Burnett[37]; Werner[38]; Merker et Gilbert[39]; et surtout Lemke[40]); nous avons retrouvé les mêmes phénomènes chez ces organismes[22], ainsi que chez certains Rotifères, dont les couronnes ciliaires sont photosensibles[19], *Brachionus pala, Hydatina senta, Asplanchna girodi*, etc. Partout, chez les animaux aquatiques dont nous avons étudié le phototropisme, la sensibilité dermatoptique assume le rôle principal, car elle est le point de départ des réactions photopositives d'approche et règle la vitesse des déplacements vers la lumière (photocinèse); les yeux, quand il y en a, contrôlent seulement l'orientation axiale précise, c'est-à-dire la rectitude des trajets. Et, dans tous les cas, la sensibilité dermatoptique nous a paru se caractériser par un accroissement graduel de l'efficacité des radiations spectrales du rouge au violet; la sensibilité visuelle présentant au contraire un maximum dans les longueurs d'onde moyennes du spectre visible (520–550 mμ).

De nombreux animaux aquatiques présentent en outre une photosensibilité diffuse, non seulement de leur tégument, mais aussi de divers tissus et organes internes. C'est notamment le cas des cellules ciliées de l'épithelium branchial de la Moule et de l'Anodonte (Viaud[18]); de diverses parties du système nerveux: chaine ventrale du Siponcle (Hertel[41]), ganglions céphaliques du Gammare aveugle *Niphargus* (Merker[55]), ganglion caudal de l'Ecrevisse (Prosser[42]; Kennedy[43],) nerfs radiaires de l'Oursin *Diadema* (Yoshida et Millott[44]), lobes optiques de la Grenouille (Koller et Rodewald[45]); du tissu musculaire également, par exemple l'iris des Vertébrés inférieurs (Arnold[46]), le coeur de la Grenouille (Amsler et Pick[47]; Sessunine[48]), le coeur de la *Daphnia magna* (Prokšova[49]). Mentionnons enfin les données de Benoît[50] sur le "réflexe photosexuel" du Canard, ou gonado-stimulation résultant d'une activation de l'hypophyse par la lumière. Mais, dans ce cas particulier, l'efficacité des radiations a son maximum dans les grandes longueurs d'onde du spectre visible (orange-rouge), la région hypothalamo-hypophysaire étant pratiquement insensible aux lumières de courte longueur d'onde.

Quoi qu'il en soit, ces faits, dans leur ensemble, montrent que tout protoplasme est *virtuellement* photosensible, même chez les Métazoaires les plus élevés en organisation, bien qu'*en réalité* cette sensibilité ait disparu de la plupart des tissus.

L'étude des comportements qui dépendent d'une part de la sensibilité dermatoptique *stricto sensu*, d'autre part de récepteurs photiques différenciés (yeux, ocelles ou simples cellules tégumentaires spécialisées et pigmentées) nous conduit à distinguer radicalement ces deux modes de la photoréception. Le sens dermatoptique ne commande jamais que des comportements phototropiques et, principalement, les aspects primaires de ces réactions: progression vers la lumière ou fuite vers l'ombre. Les yeux

ou ocelles des organismes inférieurs assurent une orientation axiale plus ou moins précise, mais ne sont pas essentiels à la manifestation du phototropisme (d'ailleurs ils font défaut chez beaucoup d'espèces phototropiques).

Quand apparaissent, dans l'évolution du psychisme animal, des comportements d'un niveau supérieur aux tropismes, en particulier des réactions perceptives, c'est-à-dire des réponses à la lumière où à l'obscurité comme à un *signe* (le phototropisme étant un ensemble de réactions à l'*agent* lumineux) elles sont toujours le fait de formations de type oculaire: c'est le cas des réactions skioptiques de la Mye des Sables qui ont leur maximum spectral à 578 mμ, tandis que leurs réactions photopiques dépendent d'autres récepteurs spécialisés, présentant un maximum de sensibilité vers 500 mμ (Hecht[51]; Koller et von Studnitz[52]). Le même phénomène a été retrouvé par Unteutsch[53] sur le Lombric: maximum skioptique en lumière jaune, maximum photopique en lumière bleue. Chez *Limnaea stagnalis*, les réactions skioptiques, tres caractéristiques, peuvent être à point de départ extra-oculaire (chez des individus aveuglés); or elles sont très probablement le fait de récepteurs spécialisés épars dans les téguments. Car elles présentent un maximum spectral qui coïncide avec celui de la sensibilité oculaire, vers 520 mμ (Medioni[54]).

Bref nous pensons que le sens dermatoptique *stricto sensu* est caractérisé *morphologiquement* par l'absence de dispositifs photorécepteurs différenciés, *physiologiquement* par une sensibilité spectrale croissante du rouge au violet et *psychologiquement* par des réponses à la lumière en tant que "stimulus-agent". Mais il existe dans la peau de beaucoup d'organismes aquatiques des récepteurs spécialisés leur conférant une sensibilité extra-oculaire que l'on peut qualifier de "para-visuelle". Ces dispositifs ne sont encore identifiés *morphologiquement* que dans un faible nombre de cas, mais se caractérisent *physiologiquement* par une sensibilité spectrale maximum dans les longueurs d'onde moyenne du spectre visible et psychologiquement parce qu'ils sont à l'origine de réactions à l'augmentation ou à la diminution de l'éclairement ambiant, perçues en tant que "stimuli-signes" (la réaction skioptique, par exemple, est une réponse à la présence éventuelle d'un prédateur).

CONCLUSIONS

(1°) Le sens dermatoptique est un mode primitif de photoréception; c'est la fonction photoréceptrice du protoplasme indifférencié quant à la photoréception, dans le règne végétal, comme dans le règne animal. (2°) A ce mode de photoréception est d'abord lié le phototropisme positif, ensuite des réactions photonégatives ou même de sensibilité différentielle, dans lesquelles la lumière est un stimulus-agent. (3°) Aux photorécepteurs différenciés et aux formations oculaires sont liées des réactions d'orientation et des réactions perceptives dans lesquelles la lumière et l'ombre sont des stimuli-signes, et dont les plus primitives sont les réactions skototélotactiques, skioptiques, les perceptions d'ombres en mouvements, etc. (4°) La vie aérienne semble avoir supprimé le sens dermatoptique, qui n'a plus subsisté alors que dans certains tissus du corps, en particulier le tissu nerveux, où il se manifeste par des phénomènes photodynamiques. Quant au phototropisme, chez les animaux supérieurs il devient exclusivement le fait des excitations oculaires.

Bibliographie p. 202

BIBLIOGRAPHIE

1 G. POUCHET, *Rev. et Magasin de Zool.*, 23 (1872) 110, 129, 183, 225, 261, 312.
2 V. GRABER, *Grundlinien zur Erforschung des Helligkeits- und Farbensinnes der Tiere*, Prag-Leipzig, 1884.
3 F. PLATEAU, *J. Anat. Physiol.*, 22 (1886) 431.
4 R. DUBOIS, *Compt. rend.*, 110 (1890) 358.
5 R. DUBOIS, *Anatomie et Physiologie comparées de la Pholade dactyle*, Paris, 1892.
6 V. WILLEM, *Bull. sci. France et Belg.*, 23 (1891) 329.
7 W. NAGEL, *Biol. Zentr.*, 14 (1894) 810.
8 W. WUNDT, *Grundzüge der psychologischen Physiologie*, 6ème éd., Leipzig, 1911.
9 H. LAURENS, *Bull. Museum Comp. Zool., Harvard College*, 53 (1911) 251.
10 M. MANQUAT, *Les tropismes dans le comportement animal*, Thèse Fac. Sci., Nancy, 1921.
11 A. FOREL, *Le monde social des Fourmis du globe comparé à celui de l'Homme*, tome II, Genève, 1921.
12 A. LAMMERT, *Z. vergleich. Physiol.*, 3 (1925) 225.
13 R. HESSE, *Bethe's Hdb. norm. pathol. Physiol.*, t. XII/1, p. 3.
14 H. PIERON, *Encyclopédie Française*, tome IV (La Vie), Paris, 1937.
15 G. VIAUD, *Recherches expérimentales sur le phototropisme des Daphnies, Étude de Psychologie animale*, Strasbourg, Paris, 1938.
16 G. VIAUD, *Le phototropisme animal*, Strasbourg, Paris, 1938.
17 G. VIAUD, *Bull. biol. France et Belg.*, 74 (1940) 249.
18 G. VIAUD, *Compt. rend. soc. phys. biol.*, 16 (1942) 128.
19 G. VIAUD, *Bull. biol. France et Belg.*, 77 (1943) 68.
20 G. VIAUD, *Experientia*, 4 (1948) 81.
21 G. VIAUD, *Le phototropisme animal, Aspects nouveaux de la question*, Paris, 1948.
22 G. VIAUD, *Behaviour*, 2 (1950) 163.
23 G. VIAUD, *Année biol.*, 27 (1951) 365.
24 N. MILLOTT, *Endeavour*, 16, nr. 61 (1957) 19.
25 S. O. MAST, *Z. vergleich. Physiol.*, 15 (1931) 139.
26 A. TREMBLEY, *Mémoires pour servir à l'histoire d'un genre de polypes d'eau douce à bras en forme de cornes*, Leiden, 1744.
27 S. DIJKGRAAF, *Zool. Anz.*, 111 (1935) 254.
28 H. SCHULZ, *Z. vergleich. Physiol.*, 7 (1928) 488.
29 J. E. HARRIS ET PEGGY MASON, *Proc. Roy. Soc. (London)*, B, 145 (1956) 280.
30 R. GINET, *Ecologie, éthologie et biologie de Niphargus*, Thèse Fac. Sci., Lyon, 1960.
31 CH. HAIG, *Proc. Natl. Acad. Sci. U.S.*, 20 (1934) 476.
32 E. BÜNNING, *Physiologie des Wachstums und der Bewegungen*, Berlin, 1939.
33 K. VON FRISCH, *Pflüger's Arch. ges. Physiol.*, 138 (1911) 319.
34 E. SCHARRER, *Z. vergleich. Physiol.*, 7 (1928) 1.
35 G. THINES, *Ann. Soc. roy. Zool. Belg.*, 84 (1953) 231.
36 D. SCHEFFER, P. ROBERT ET J. MEDIONI, *Compt. rend. soc. biol.*, 152 (1958) 1000.
37 G. H. PARKER ET F. L. BURNETT, *Am. J. Physiol.*, 3 (1900) 271.
38 O. WERNER, *Zool. Jahrb. allg. Zool. Physiol.*, 43 (1926) 41.
39 E. MERKER ET H. GILBERT, *Zool. Jahrb. allg. Zool. Physiol.*, 51 (1932) 441.
40 G. LEMKE, *Z. vergleich. Physiol.*, 22 (1935) 298.
41 E. HERTEL, *Z. allgem. Physiol.*, 6 (1906) 44.
42 D. L. PROSSER, *J. Cellular Comp. Physiol.*, 4 (1934) 185.
43 D. KENNEDY, *Am. J. Ophthalmol.*, 46 (1958) 19.
44 M. YOSHIDA ET N. MILLOTT, *Experientia*, 15 (1959) 13.
45 G. KOLLER ET W. RODEWALD, *Pflüger's Arch. ges. Physiol.*, 232 (1933) 637.
46 F. ARNOLD, *Physiologie*, 2 (1837).
47 AMSLER ET PICK, *Arch. exptl. Pathol. Pharmakol., Naunyn-Schmiedeberg's*, 82 (1917) cité d'après, VON STUDNITZ[56].
48 P. A. SESSUNINE, *Bull. biol. méd. exptl. U.R.S.S.*, 1 (1936) cité d'après VON STUDNITZ[56].
49 MILADA PROKŠOVA, *Acta Acad. Sci. Natural, Morova-Silesiacae*, 22 (1950) 34.
50 J. BENOÎT, *Compt. rend. soc. biol.*, 127 (1938) 906.
51 S. HECHT, *J. Gen. Physiol.*, 3 (1921) 367.
52 G. KOLLER ET G. VON STUDNITZ, *Z. vergleich. Physiol.*, 20 (1934) 388.
53 W. UNTEUTSCH, *Zool. Jahrb. allg. Zool. Physiol.*, 58 (1937) 69.
54 J. MEDIONI, *Compt. rend. soc. biol.*, 152 (1958) 840.
55 E. MERKER, *Verhandl. deut. Zool. Ges.*, 33e session (1929) 157.
56 G. VON STUDNITZ, *Physiologie des Sehens*, Leipzig, 1940.

PERIODIC CHANGES IN THE VISUAL PIGMENT OF A FISH

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Mr. Chairman, Ladies and Gentlemen

The investigation I am to talk about this morning began, as so many do, from a chance observation. In the winter of 1959 we were examining the visual pigments of as many British fresh-water fish as we could lay our hands on. Among the fish we received from our supplier at this time was a consignment of rudd (*Scardinius erythrophthalmus*). This is a common fish in Britain characteristic of lakes and sluggish rivers.

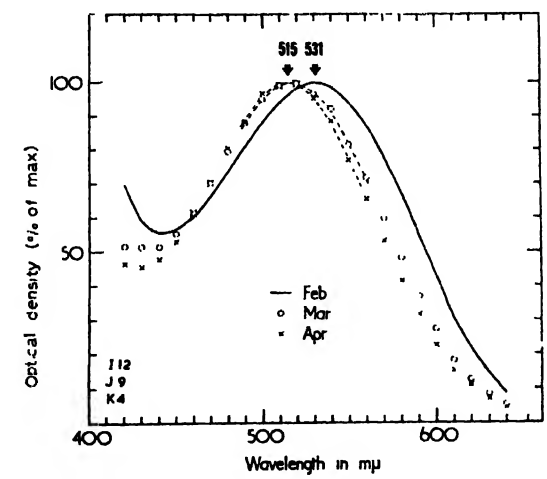

Fig. 1. Variability of the rudd visual pigment. Continuous line, absorption (optical density) spectrum of extract (I 12) made on Feb. 3rd 1959 by method 1 (see text) from a consignment of fish. Circles, extract (J 9) made on Mar. 19th by method 2 from same consignment. Crosses, extract (L 4) made on April 7th by method 1.

An extract of the visual pigment was prepared from the retinae of some of these rudd and its absorption spectrum is shown by the continuous line Fig. 1. This spectrum seemed to be in no way remarkable. It had a maximum at 531 mμ, and we at first supposed that it contained visual pigment 533, a pigment which is found in a number of fresh-water fish. We did not, therefore, examine the extract more closely at this stage.

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References p. 213

About six weeks later, we had occasion to make another extract from the same sample of rudd. To our surprise the absorption spectrum, shown by the circles in Fig. 1, was quite different from that obtained before. We thought at first that this difference might be due to the fact that we had used slightly different procedures for extracting the visual pigment in the two experiments. Thus, the first had been prepared simply by allowing whole, washed retinae to stand in digitonin solution. The second extract, however, was made from the visual cells alone, after these had been separated from the rest of the retina by floating them in 35% sucrose solution. Accordingly, to settle whether the differences were due to procedural variation, we made yet a third extract, shown by the crosses in Fig. 1, but this time using the first method. As you see we obtained very nearly the same result as on the second occasion, showing that the procedure used in extraction was not the cause of the differences observed. The real cause of these differences is connected with the lapse of time since we had first received the fish. In anticipation of what I shall deal with in detail later on, I may say now that the visual pigment of the rudd is not constant but varies according to the time of the year and also according to the conditions under which the fish is kept.

Now how is it possible for a visual pigment to vary in its light-absorbing properties? It would seem very unlikely that there could be an actual change in light-absorbing properties of a single pigment. A much more probable explanation would be that there is more than one pigment present in the rudd retina and that the changes are *due to variations in the proportions of the pigment*. Our task, therefore, was to try to *determine the composition of the visual pigment extracts*: to ascertain whether they were *homogeneous*, or consisted of a *mixture* of pigments and, if the latter, what the proportions of the various pigments were.

The composition of a visual pigment extract can be determined by the method of partial bleaching(Dartnall[1,2]). As the name implies, in this method the extracts are partially bleached with monochromatic lights drawn from different parts of the visible spectrum. Now if an extract contains only one pigment then clearly the changes from stage to stage in a series of partial bleachings must be qualitatively the same. If, however, the extract is a mixture, then by suitable choice of the bleaching wavelength it should be possible to affect one pigment more than another. In this event the changes from one stage to the next will be qualitatively different.

Accordingly we carried out a large number of partial bleaching experiments by which we ascertained that in every one of the rudd extracts we prepared — and there were more than forty in all — there were two visual pigments and two pigments only. The properties of these two pigments were constant. The marked variation in the properties of the whole extracts were due to variations in the relative *amounts* of these two constituent pigments.

As an example of this let us consider Fig. 2. In the upper part of the Figure are shown the absorption spectra (first curves through white circles) of two extracts which had quite different light-absorbing properties; one of them showing a maximum at 517 mμ and the other, a maximum at 529 mμ. When these extracts were exposed to deep red light of a certain intensity for 2 h, they were partially bleached to the stages shown by the second curves through the white circles. Previous experiments had shown us that this deep red light — actually of wavelength 660 mμ — was effective in bleaching one of the constituent pigments in the extracts without markedly

affecting the other. A further 2 h exposure to the same red light caused little further change (first curves through black circles), confirming that nearly all of the red-sensitive pigment had been removed by the first bleach and also that this light had little effect on the remaining pigment. Finally, on exposing the extracts to white light (15-W lamp, 10 min), considerable further changes took place (second curves through black circles). These were due to the bleaching of the red-insensitive components of the extracts.

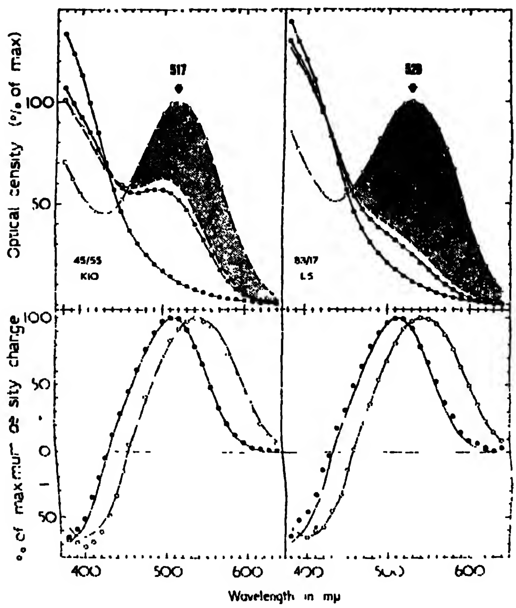

Fig. 2. Variability of the rudd visual pigment is due to alterations in the relative proportions of two constant pigments. Upper half of figure shows the absorption (optical density) spectra of two different extracts (with absorption maxima at 517 and 529 mμ respectively) and the results of partially bleaching them, first with 660 mμ light for 2 h, then for a further 2 h, and finally with white light. Lower half of figure shows that the difference spectra for the first red bleaches (white circles) and for the final white bleaches (black circles) are practically identical in the two extracts showing that the same two pigments are present but in different proportion. The pure difference spectra (see text) for the red sensitive and red-insensitive pigments of the rudd retina are shown by the continuous curves.

The difference spectra for the first red bleaches and for the final white bleaches are shown in the lower part of Fig. 2 by the white and black circles respectively. The continuous curves you see are not drawn through these results but are difference spectra for the pure constituent pigments of rudd extracts. The agreement of these curves with the results shows that the red-sensitive and red-insensitive components of these two quite different extracts are qualitatively identical. It is only their proportions which vary. Thus the extract "K 10" with maximum at 517 mμ contained about as much red-sensitive pigment (shown by the hatching) as red-insensitive pigment, the actual proportion being 45% of the former and 55% of the latter. The extract "L 5", with a 529-mμ maximum, however, was very rich in the red-sensitive component, having the composition 83/17.

Now, of course, it is never possible to effect a perfect separation of pigments in a mixture by partial bleaching. The red light must bleach some of the red insensitive

References p. 213

pigment. Perhaps I should say a few words, therefore, about how we can decide the true values for the difference spectra. Now it is a fairly easy matter to ensure, by many exposures of an extract to deep red light, that every trace of red-sensitive pigment is removed. By subsequent bleaching of the red-insensitive pigment we can, therefore, obtain its true difference spectrum. With extracts which have been denuded of their red-sensitive component in this way we can also find what small proportion of the remaining insensitive component is bleached by the deep red light. With this knowledge the difference spectrum for any red bleach can be corrected for the contribution of the red-insensitive pigment which it includes. When this is done it is found that all difference spectra obtained by red-light bleaching agree.

Fig. 3 shows the pure difference spectra for the two pigments present in every rudd retina. The white circles refer to the red-sensitive pigment and the black circles to the red-insensitive pigment.

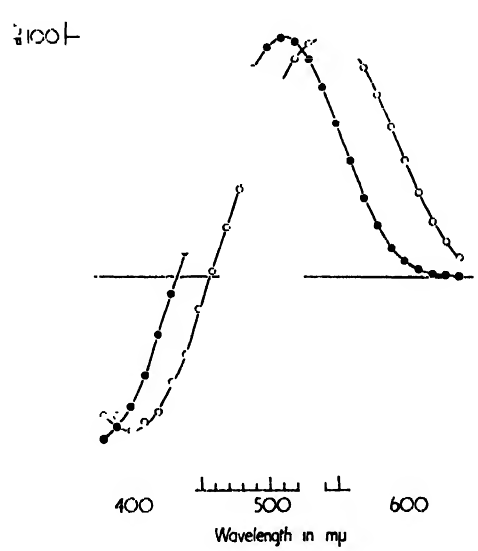

Fig. 3. The difference spectra of the two retinal pigments of the rudd, visual pigment 543 (white circles), a vitamin-A_2 pigment, and visual pigment 510 (black circles), a vitamin-A_1 pigment.

Now a difference spectrum is the difference between the absorption spectrum of a visual pigment and that of its product of bleaching. Such functions have a positive portion which approximates to the absorption spectrum of the pigment and a negative portion which approximates to that of the product. The red-sensitive pigment (white circles) has a maximum at about 543 mμ and bleaches to a product having its maximum at about 400 mμ. This indicates the product to be retinene$_2$ and the pigment 543 to be a vitamin-A_2 pigment. The red-insensitive pigment has a maximum at 510 mμ and, from the nature of the negative portion of its difference spectrum, appears to be a vitamin-A_1 pigment.

From the two curves shown in Fig. 3 it is possible to construct any number of intermediate curves to represent mixtures of the two. Armed with a series of these curves we could then determine the composition of any difference spectrum by finding which constructed curve it matched. The way we used this principle is shown in Fig. 4.

In this figure the ordinates are wavelength in $m\mu$, and the abscissae the percentage of the 543 pigment in the mixture. Let us first consider the curve labelled "line of λ_{max}".

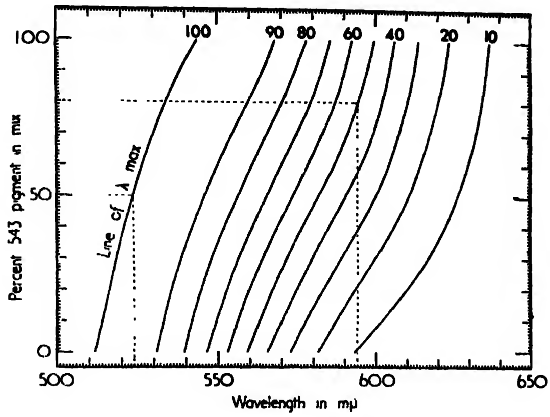

Fig. 4. Chart for determining the composition of a mixture of visual pigment 543 and visual pigment 510. For explanation see text.

This shows the relationship between the composition of a mixture and the maximum of its difference spectrum. For example, a 50/50 mixture has a maximum at 524 $m\mu$. Similarly, the other lines marked 90, 80 and so on, give a like relationship between the composition and the ordinate of the long wave-length arm of the difference spectrum. For example, the difference spectrum of a mixture consisting of 80% pigment 543 has a 50% point at 594 $m\mu$.

Given any difference spectrum, therefore, we can obtain a number of estimates for its composition, namely from its maximum, a 90% point, an 80% point and so on. The mean of these estimates gives a very reliable value.

A proof of the accuracy of this method is provided by the fact that estimates of composition from the overall difference spectrum always agreed – usually to better than 2% – with the estimates obtained by adding together the values obtained from the individual difference spectra.

Having made the observation that the visual pigments of the rudd changed over a period of time in our aquarium, we anxiously enquired about further supplies. Anyone who has worked in this field knows how exasperating it is when the supply of an interesting animal just dries up. But fortune smiled on us. We learned from our supplier that he had about 2000 of these rudd, in a small pond all by themselves, that they all had the same origin and history, were all the same age and size and were at our disposal. Accordingly, we were able to plan an extensive series of experiments which continued for well over a year and which consumed some 400 of these rudd.

Our next consignment of rudd was divided into two equal batches. One half of them was put into an outside aquarium ("normal"), where the conditions were brighter than in the pond from which they came. The other half was put into a dark-room aquarium ("dark"). At intervals we took samples from both these batches and examined the composition of their visual pigments. The results are shown in Fig. 5. In this, and in Figs. 6 and 7 the pattern of experimentation was the same. The absorption spectrum of the extract was determined. It was then bleached for 2 h with deep red light; then for a further 2 h with the same red light; and finally with white light. The

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changes due to the first red bleach are shown shaded. In the top left hand corner of Fig. 5 are shown the results for the rudd "K 8" as received from the pond. They contained a mixture of the 543- and 510-pigments in the proportions 71/29. After 13 days in the outside aquarium ("K 10") this composition had changed quite dramatically to 45% pigment 543 and 55% pigment 510; while after 46 days ("L 6") the situation had so advanced that the extract which was originally very rich in the 543 pigment was now very rich in the 510 pigment. Now let us see what happened to the

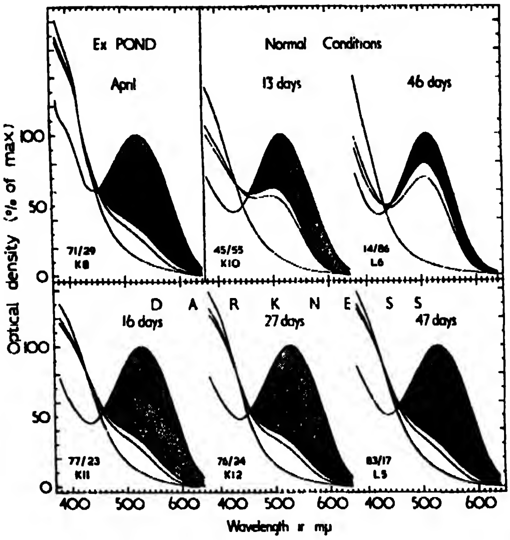

Fig. 5. Effect of light environment on the relative proportions of the pigments 543 and 510 in the rudd retina. Material bleached by first exposure to red light shown shaded. Then followed a second exposure to red light and finally an exposure to white light. The proportions of the 543 and 510 pigments are shown thus: 71/29 - 71% 543, 29% 510. "Normal conditions" = brighter than in natural habitat, *viz.* in an open aquarium with side window, and subjected to the normal alternation of day and night.

fish that were kept in the dark room. There was less change with these, though in 47 days the 543 pigment rose from the original 71% to 83%. We may conclude that putting the fish into darkness causes the proportion of 543 pigment to increase. Conversely, putting them into a bright environment causes the proportion of the 510 pigment to increase.

Fig. 6 demonstrates that these changes are reversible. The curves in the upper half of the figure show how, after some fish had spent 13 days in the outside aquarium, the 543 pigment fell from its original 71% to 45%. After 20 days in this environment some of these fish were transferred to the dark room aquarium and after 15 days there, the 543 pigment had recovered to 66%.

In the lower part of Fig. 6 is shown the converse experiment. After some fish had been 16 days in the dark room aquarium, the 543 pigment rose from its original 71% to 77%. After 19 days in darkness some of these fish were transferred to the outside aquarium and after 15 days there the 543 pigment had fallen to 53%.

I have spoken as though the visual pigment situation of fish can be examined at intervals. This, of course, is true only in a certain sense. The examination of the visual pigments of a fish by our methods necessarily involves killing it, and what in fact one does is to examine a sample of a large population at intervals; and one has to

assume that each sample is truly representative of the whole. This, I think, is a fairly reasonable assumption, not only because all the fish were of the same age and size and had the same history, but because 10 fish were used in preparing each extract, a number sufficient to iron out any individual variations.

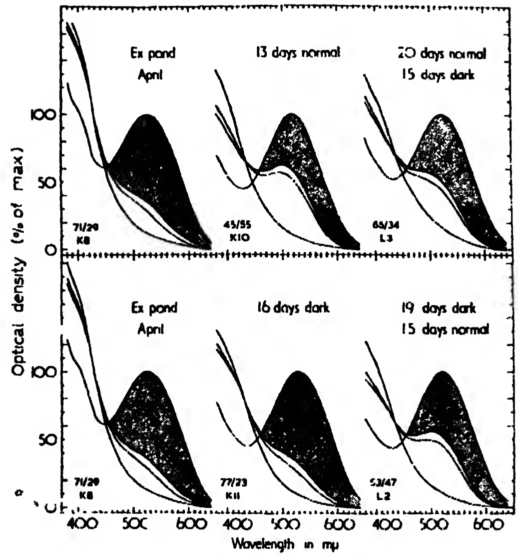

Fig. 6. Reversibility of the changes caused by light environment to the proportions of pigments 543 and 510 in the rudd retina. Terms and layout as for Fig 5.

In Fig. 7 are shown some more results. In the upper half of the figure a similar experiment to that in the upper half of Fig. 6 is displayed, but with some fish received in May ("L 4"). These contained a 49% proportion of the 543 pigment compared with the 71% of the April fish ("K 8", Fig. 6). After 20 days in the outside aquarium the 543 pigment fell to 19% but this trend was reversed by putting the fish in the dark room for, after 14 days there, the 543 pigment had recovered to 46%.

The results in the lower half of Fig. 7 are of special interest. You may imagine that with batches of fish being received at periodic intervals and with each batch the subject of experiments in different light environments, the pressure on our dark room space became insupportable. Because of this we decided to black out one of our outside aquaria. This we did by pasting black photographic paper over the front window and by resting on the top of the aquarium a piece of thin mild steel sheet to exclude top light. These arrangements were not absolutely light tight, for there were tiny chinks left between the top cover and the sides of the aquarium, which allowed a little daylight to enter. After some fish (extract "L 11") had spent 28 days in this aquarium ("normal dim") the 543 pigment *fell* from the original 49% to 39%. We had expected a rise in these conditions of near darkness. When some of these fish were transferred to the dark room for 44 days (extract "M 9") the 543 pigment showed the expected rise, actually to 68%. This showed that the fish were behaving normally. The unexpected result obtained with the fish which had been kept in the inadequately blacked out aquarium worried us. We found it difficult to believe that the very dim conditions inside this tank could be so different to the fish from complete darkness as to reverse

References p. 213

the expected trend. In this we were quite wrong. Not really believing that it would make any difference we went to great trouble to tape up every possible light leak in the tank. To our surprise and relief the pigment 543 then began to accumulate in the rudd retina and, after 20 days, had risen from 39% to 55%.

In Fig. 8 I have endeavoured to summarise our experiments. Consider first the

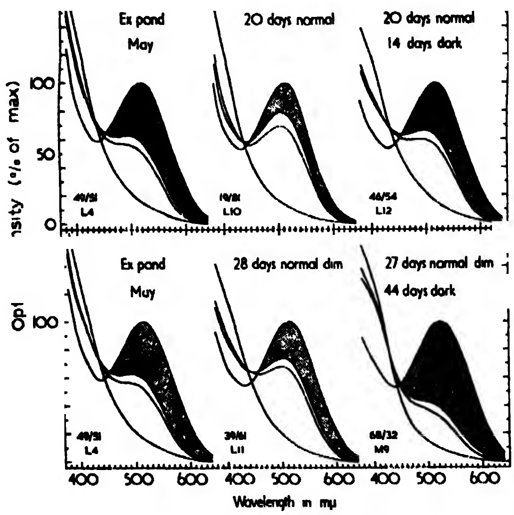

Fig. 7 Further experiments on the effect of light environment on the proportions of pigments 543 and 510 in the rudd retina. For explanation see text.

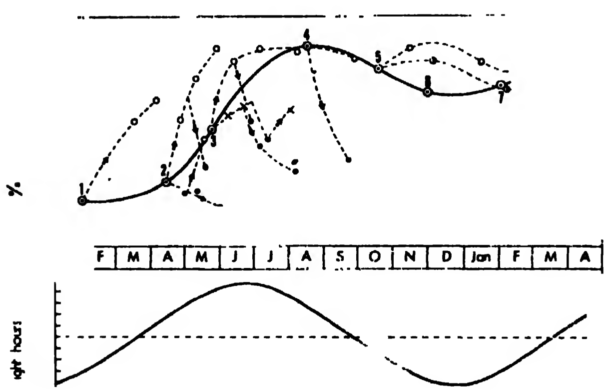

Fig. 8. Upper half, summary of experiments (fish unfed except for those of consignment 5). For explanation, see text. Lower half, the sunrise-sunset interval for latitude 52°N from *The Abridged Nautical Almanacs* for the years 1959 and 1960, London, Her Majesty's Stationery Office.

upper part of the figure. This represents a plot of the percentage of the 510 pigment in the retinal mixture as a function of the date. The circles labelled 1 to 7, give the visual pigment compositions of the 7 consignments of fish as received from the pond between January 1959 and April 1960. The curve through these circles is not dissimilar to the curve shown in the bottom part of the slide. This is the daylight-hours curve for latitude 52°N as given by the Nautical Almanac. Thus in the winter months when the

days are short the 510 pigment is minimal while in the summer months it is maximal. There is a difference in amplitude of pigment variation as between 1959 and 1960 which we cannot explain. Whether there was a change in the general brightness level between these years or whether the amplitude difference depended on factors of growth in the rudd — which were, of course, 15 months older at the end of our experiments — we are unable to say.

The other points which are joined directly or indirectly to the numbered circles represent the results of experiments which were done on that particular consignment of fish. The white circles represent results from fish which had been kept in our outside tanks, *i.e.*, subjected to the normal alternation of day and night. The black circles give the results on fish which had been kept in darkness. I will run briefly through the experiments. Of the first consignment samples were examined on arrival to give the point 1 and again after two periods in an outside tank. A similar experiment was done with the second consignment and you will observe that, as before, the proportion of pigment 510 increased on placing the fish in the bright outside tanks. After spending some 20 days in this tank some fish of the second consignment were placed in darkness with consequent abrupt change in pigment composition. Conversely, after about 20 days in the dark room, some fish of this same consignment were transplanted to the outside tank with a similar but opposite change.

The results of the experiments with the third consignment are particularly interesting. Again, some fish were put in the outside aquarium with consequent rise in the proportion of pigment 510 towards an asymptotic value of about 85%. After 20 days some of these were transferred to the dark room causing a fall in this proportion. The crosses represent the results obtained with some of this consignment which were put into our inadequately blacked out aquarium. The movement of pigment composition in this case was, as I mentioned earlier, in the opposite direction to that expected. When, after 20 days, some of these fish were put in the dark room the proportion of 510 pigment fell. After 35 days in the inadequately blacked out tank, all light leaks were carefully blocked with tape. There was then an immediate change in composition in the expected direction. On reverting to the inadequate condition the direction of this change was reversed.

The experiments with the fourth consignment were interesting because in these the original proportion of the 510 pigment was at its maximum of about 85%. Consequently, on placing some of them in darkness a very large fall in the 510 proportion occurred and a correspondingly large rise in the 543 proportion. In the outside tank, however, no rise, indeed a small fall in the 510 proportion took place. It seems that there are natural limits to the proportions, that of the 510 pigment never falling below 15% nor rising above 85% — and conversely for the 543 pigment — whatever the time of year or treatment undergone by the fish.

The results with the fifth consignment require a special word of explanation. The open circles represent, as before, the results obtained with fish which had been put in the outside aquarium. The half filled circles, on the other hand, represent results on fish which had been put into an outside aquarium which had its front window blocked and its interior painted black. The initial rises in pigment 510 in both aquaria were probably due to the fact that they were both brighter than the original pond. The subsequent falls are attributable to the shortening of the days and the final rises to their lengthening.

References p. 213

Up till now I have given you the results of our experiments in the form of proportions of the two pigments. I have said nothing about quantities. In fact, all our experiments were carried out under strictly identical conditions so that the results we obtained were comparable on a quantitative basis. I would like in the few moments

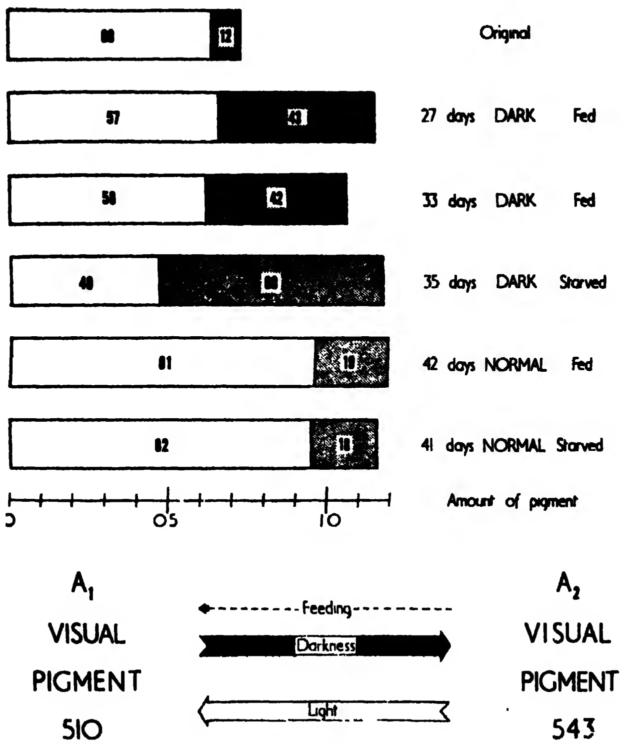

Fig. 9. Quantitative changes in the amounts of the 543- and 510 pigments in the retina of the rudd after periods of starving or of feeding under normal conditions (*i.e.* subject to the alternation of day and night) and in darkness. Rudd of 4th consignment. For explanation see text.

left to me to direct your attention to a particular sequence of quantitative experiments that we carried out. These are illustrated in Fig. 9 which shows the absolute amounts of the two pigments present in a consignment (the fourth) of rudd at the beginning, *i.e.* as received from the pond, and also after the lapse of periods of time during which the fish had been subjected to various treatments. The lengths of the white and shaded rectangles are proportional to the absolute quantities of the 510- and 543 pigments respectively, a density scale being provided in the middle of the figure.

First a word about the treatment the fish received. In all the previous experiments I have described (except those done on the 5th consignment), the fish were not fed after receipt from our supplier. In the present experiments, however, the effects of feeding and of starving were investigated both in dark and in outside aquaria.

The original proportion of pigment 510 in the fourth consignment was 88%. This proportion diminished in fish which had been kept in darkness whether they were fed or starved, but was more marked in the starved fish. The food was crushed dog biscuit, which probably contains some vitamin-A_1 but which is unlikely to contain any vitamin-A_2. It is readily understandable therefore that feeding would reduce the rate at which the 510 pigment diminishes in darkness. The fish kept in the outside tank did not suffer much change in visual pigment composition and no difference could be

detected between the fed and the starved batches. This may be due to the fact that the consignment originally possessed the maximum 510-pigment proportion and consequently there was no scope for any increase.

Now I would like to consider the absolute quantities of the pigments. Originally the total amount of pigment was about 0.7 (in terms of the density scale shown in Fig. 9), while after some 30 40 days it had risen to between 1.1 and 1.2. This rise in absolute amount of pigment on keeping fish in any of our aquaria occurred in all but one of our experiments and I am at a loss to explain it. It may be that London tap water is more supercharged in dissolved gases than is the natural pond water. Certainly anyone who has ever left a tumbler of water on his dressing table overnight in London will have noticed that by morning there are innumerable bubbles clining to the side of the glass, showing that the water was originally supercharged in air. Granting this inexplicable increase in the amount of pigment, the particular interest in Fig. 9 is that at the end of the testing period the total amount of pigment was approximately constant, irrespective, and this is the point I wish to emphasise, irrespective of the treatment the fish had received or the final proportions of the two pigments.

The fact that the amount of total pigment is approximately the same in all cases even though the proportions vary greatly, suggests very strongly that one pigment is formed at the expense of the other and *vice versa*, depending on the conditions. This I have illustrated in the lower part of Fig. 9, which suggests that in darkness the vitamin-A_1 pigment 510 is converted into the vitamin-A_2 pigment 543, and conversely in the light.

As to the mechanism of interconversion between vitamin-A_1 and vitamin-A_2 pigments we can say nothing at this stage. A fascinating ecological point is, why does the rudd change its pigments? The 543 pigment would be well suited for the detection of long wave light; why should this pigment preponderate in winter, and the other pigment, more suitable for the detection of short wavelengths, preponderate in summer? Does the transmissivity of water change between summer and winter? Does the colour of the vegetation on which the rudd feeds change? Are the visual pigment changes anything to do with the sex life of the fish?

Here is a further question. Are the two visual pigments of the rudd contained in separate visual cells or do they occur mixed in the same cells? Our guess is that they occur mixed and that the 543- and 510 pigments use the same "opsin".

This investigation has certainly raised more questions than it has answered. There were times when we felt we were battling with a Hydra of mythology — no sooner did we cut off one head than two more appeared in its place.

And now on this hot August morning I will leave you with a chilling thought. What if we had made only one extract from the rudd at a time when its retina was rich in the vitamin-A_2 pigment 543? And what if someone else, then also made a single extract — but at a time when the rudd retina was rich in the vitamin-A_1 pigment 510?

ACKNOWLEDGEMENT

We record with pleasure our thanks to Mr. D. F. Leney for supplying the fish used in these investigations and for his co-operation at all times.

REFERENCES

[1] H. J. A. DARTNALL, *J. Physiol.(London)*, 116 (1952) 257.
[2] H. J. A. DARTNALL, *The Visual Pigments*, Methuen, London, John Wiley, New York, 1957.

POLARIZED LIGHT ORIENTATION BY AQUATIC ARTHROPODS*

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This report reviews our recently completed and current experiments on the polarized light orientation of aquatic animals. To begin with, it can be definitely stated that in a variety of arthropods from three major classes of the phylum the plane of polarization of linearly polarized light is perceived by some visual mechanism different from that which senses light intensity patterns. The same conclusion now seems likely for cephalopods, too[1-3].

The reassertion that polarized light is seen as such by these animals seems necessary because considerable currency has been given to the notion that responses to polarized light are mainly, if not entirely, mediated by light intensity patterns established by differential scattering, refraction and reflection[4-7]. Since phototactic responses could occur to horizontal light intensity patterns arising from differential scattering of polarized light by particles in the medium, this idea is not unreasonable for free-swimming animals[8]. However, more detailed study of the relation between turbidity and the polarized light responses of the crustaceans, *Daphnia* and *Mysidium*, demonstrated a

TABLE I ORIENTATION ASYMMETRY IN *Daphnia schodleri* SWIMMING IN A VERTICAL BEAM OF LINEARLY POLARIZED LIGHT (modified from Waterman[9])

Animal	*Run*[a]	*Medium*	*Orientation relative to e-vect*
1	1	Clear	120°
	2	Clear	120
	3	Clear	105°
	4	Turbid	90°
	5	Turbid	105°
	6	Turbid	105°
2	7	Turbid	105
	8	Turbid	75°
	9	Turbid	90°
	10	Clear	75°
	11	Clear	75°
	12	Clear	75°
3	13	Clear	60°
	14	Clear	60°
	15	Clear	60°
	16	Turbid	60°
	17	Turbid	60°
	18	Turbid	60°

[a] Each run consisted of 120 measurements of the animal's azimuth orientation.

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...plex interaction between turbidity and polarized light such that light intensity ...tterns are quite unlikely to account for the observed orientation[9].

Further study of these and three other aquatic arthropods sustained this conclusion and provided several new types of data whose only explanation is that there are two distinct visual input channels, one for plane of polarization and another for intensity patterns[2]. For example, reversing the phototactic sign of the dytiscid beetle *Bidessus* and of *Daphnia* has no effect on their polarized light responses although their light pattern responses are reversed as expected. Our experiments and conclusions are quite in agreement with those of von Frisch, Lindauer and Daumer[10] who have presented extensive new proof that polarized light perception by the honey bee is a distinct physiological process.

Quantitative study of the orientation of *Daphnia* and *Mysidium* in vertical beams of linearly polarized light has shown that the swimming direction relative to the *e*-vector is not arbitrarily fixed and may change significantly from time to time[9]. Thus the previously described predominance of swimming perpendicular to the polarization plane is only a rough approximation. Although these crustaceans may swim at a constant angle to the *e*-vector most of the time, this angle may at any particular moment differ by as much as $\pm$ 30° from the perpendicular (Table I).

Furthermore, such perpendicular or nearly perpendicular orientation relative to

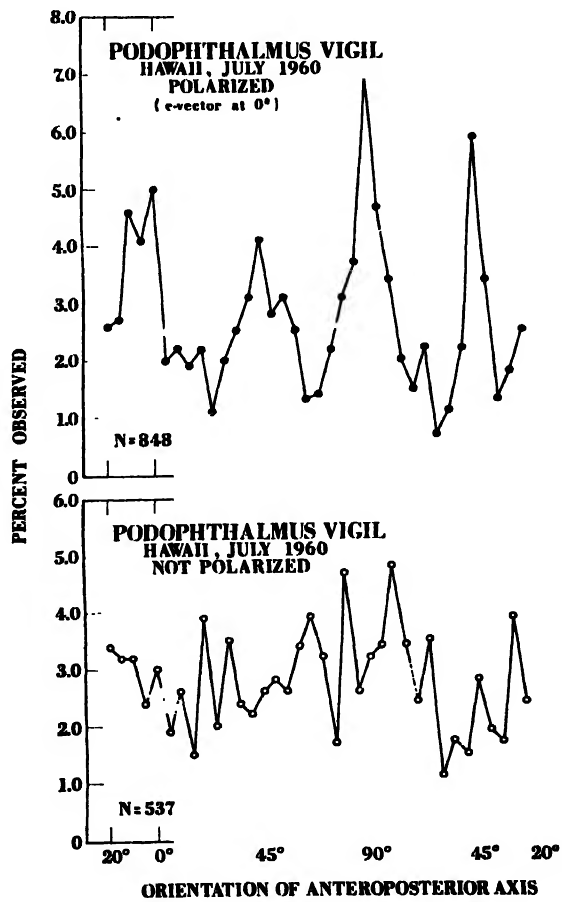

Fig. 1. Directional orientation of the brachyuran crab *Podophthalmus vigil* in a vertical beam of white light. In the upper part of the figure the vertical beam is linearly polarized and the animals clearly showed preferential orientation at 0°, 90° and $\pm$ 45° relative to the *e*-vector. In the lower part of the figure the vertical beam was not polarized and no evidence for these four basic directions is obvious. The numbers of observations are indicated by the N's in the figure

References p. 216

the *e*-vector may result from a special stimulus situation in which three other preferential swimming directions are suppressed by strongly directional illumination *(light contrast reaction)* and high overall light intensities *(light intensity effect)*[2]. Under appropriate lighting conditions *Daphnia*, the amphipod *Hyalella*, *Bidessus* and the mite *Arrenurus* orient preferentially in four basic directions parallel, perpendicular, and $\pm$ 45° to the *e*-vector of a linearly polarized vertical light beam[2].

The same four basic orientation directions have also been found in many decapod crustaceans (Natantia and Brachyura including certain larval stages, juveniles and adults) (Fig. 1), stomatopods and two species of squid[3]. The widespread occurrence of these basic swimming directions suggests that such orientation could be an important component of the menotaxis of the aquatic animals concerned. The relation of these patterns of spatial orientation to the natural radiance distribution under water and to the submarine polarization pattern has been reviewed elsewhere[11].

The closely similar manner in which a wide variety of arthropods and two distinct kinds of cephalopods respond in a vertical beam of polarized light draws attention again to the problem of mechanism which remains largely unsolved. Apparently this cannot reside in the gross morphology of the eyes since these range all the way from relatively simple cup-like ocelli in mites to the curious cyclopean eye of *Daphnia*, to the compound lateral eye of insects and malacostracan crustaceans[12], to the classic camera-type eye of cephalopods.

The most general structural correlate of polarized light sensitivity is the presence of ultramicroscopic tubules in the rhabdom-like elements of the retina[13,14]. These tubules have their long axes perpendicular and radial to the optical axis of the retinal cells of which they form a part and are probably the site of the photoreceptor pigment in such eyes[15]. As in plant cells[16], dichroism in regularly arranged molecules of this visual pigment would seem to be the most likely hypothesis for the underlying mechanism of polarized light perception. But much remains to be done before this idea can be adequately tested.

REFERENCES

1 M. F. MOODY AND J. R. PARRISS, *Nature*, 186 (1960) 839.
2 R. JANDER AND T. H. WATERMAN, *J. Cellular Comp. Physiol.*, 56 (1960) 137.
3 T. H. WATERMAN, R. JANDER AND K. DAUMER, unpublished, 1960 (Work carried out at the University of Hawaii Marine Laboratory).
4 H. DE VRIES AND J. W. KUIPER, *Ann. N.Y. Acad. Sci.*, 74 (1958) 196.
5 E. R. BAYLOR, *J. Exptl. Biol.*, 36 (1959) 369.
6 H. KALMUS, *Nature*, 184 (1959) 228.
7 F. E. SMITH AND E. R. BAYLOR, *Ecology*, 41 (1960) 360.
8 R. BAINBRIDGE AND T. H. WATERMAN, *J. Exptl. Biol.*, 35 (1958) 487.
9 T. H. WATERMAN, *Z. vergleich. Physiol.*, 43 (1960) 149.
10 K. VON FRISCH, M. LINDAUER AND K. DAUMER, *Experientia*, 16 (1960) 289.
11 T. H. WATERMAN in: *Symposium on Radiant Energy in the Sea*. Int. Assoc. Phys. Oceanog., Helsinki, 1960, in the press.
12 T. H. WATERMAN in. T. H. WATERMAN (Editor), *The Physiology of Crustacea*, Academic Press, New York, 1961, Vol. II, Chap. 1, pp. 1–64.
13 H. FERNÁNDEZ-MORÁN, *Rev. Mod. Phys.*, 31 (1959) 319.
14 M. F. MOODY AND J. D. ROBERTSON, *J. Biophys. Biochem. Cytol.*, 7 (1960) 87.
15 G. WALD AND R. HUBBARD, *Nature*, 180 (1957) 278.
16 L. F. JAFFE, *J. Gen. Physiol.*, 43 (1960) 897.

Symposium 4

LUPUS VULGARIS

Chairman: P. V. MARCUSSEN, Copenhagen, Denmark

Secretary: Sv. A. KVORNING, Copenhagen, Denmark

LUPUS VULGARIS

L. VAN DER LUGT

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The modern treatment of lupus vulgaris with INH gives excellent results. As in every treatment with antibiotics, it is to be feared that the bacilli become resistant. However in the case of the lupus vulgaris there appears to be no proven case of resistance described in the literature.

In one patient I found no reaction to the INH. The biopsy before the treatment was negative. After some months of treatment a second biopsy was performed. In this case tubercle bacilli were cultured, which were inhibited in their growth at 5 μg for each cm^3 of medium. The same figure was found by Meyer-Rohn before he started treatment in one case. This seems to be exceptional and also my patient seems to have been resistant to INH from the start. All other patients who had relapses after a course of INH, reacted promptly to a second course, and the growth inhibition was as before at 0.05–0.15 μg for each cm^3 of medium.

For the lupus the resistance to INH seems to be exceptional but when the patient has some other form of tuberculosis, combined treatment with P.A.S. or (and) streptomycin should be given.

In 1958 Dr. Rottier and I published a study[1] on the working mechanism of the Finsen-therapy which made it clear that without the rays that can form vitamin D in the skin, the Finsen-lamp has no effect at all. There are other arguments for the idea that the Finsen-lamp works by means of vitamin D formation. Lomholt[2] showed that in healing lesions of lupus vulgaris treated with the Finsen lamp, activation of the fibroblasts appears. The same phenomenon is found in healing lupus lesions of patients who receive vitamin D by mouth, and also in the tissue culture of fibroblasts if vitamin D is added.

Charpy based the vitamin D therapy on the fact that vitamin D is the factor common in the light and diet therapies, which were used previously. All this emphasizes the importance of the work of Finsen. The fact that you can prove that the Finsen-therapy is a form of vitamin D therapy takes us one step forward, but only one, as the working-mechanism of the vitamin D is not yet clear. Several facts must be borne in mind, when an attempt is made to explain the working of the vitamin D.

(*a*) Vitamin D injected locally in the lupus lesion is effective (Jensen[3]).

(*b*) Vitamin D in the dosage used in lupus vulgaris can very definitely damage any patient with pulmonar tuberculosis. This bad effect on tuberculous diseases of the lungs has been very distinctly demonstrated by Ridderbos[4]. He used high dosages of vitamin D in a great number of patients with bronchitis tuberculosa. This process

References p. 220

itself, which is comparable to the lupus vulgaris, reacted very well, but the lung process flared up in so many cases that it could not be a coincidence.

The hypothesis of Klip[5] seems at present the best. Klip states that vitamin D works on the phospholipid metabolism of the tubercle bacilli: when this happens, the tissue defence mechanism can better attack the bacilli. This will happen in the skin. The skin and subcutis are not damaged, as they have no phospholipid metabolism. In the lung the tissue has a strong phospholipid metabolism and can therefore be damaged. It is thus understandable, why in a number of cases the tuberculous processes flare up.

The experiments of Stringer seem to agree with this theory. When he gave guinea pigs vitamin D by mouth, granuloma artificially induced by phthioic acid (a synthetic product of the tubercle bacilli, belonging to the group of the phospholipids) disappeared more rapidly than in the control animals. Some experiments which I made seem to agree with Stringer's experiments. A group of guinea pigs were all treated with BCG suspension subcutaneously. Half of them received vitamin D (*ca.* 20,000 IU) a day by mouth for 6 weeks and in these weeks 2 doses of 300,000 IU intramuscularly. An intracutaneous injection with BCG suspension gave in the vitamin D-treated animals only an infiltrate, whereas the control animals developed an ulcer. The tuberculin sensitivity was also less in the vitamin D-treated animals than in the control animals.

One experiment remains to be mentioned. Four guinea pigs treated with vitamin D and BCG suspension as mentioned above, received a suspension of human tubercle bacilli intracutaneously after 6 weeks. The vitamin D animals developed an infiltrate and the controls an ulcer. Ten days later they were given tuberculin intracutaneously. The vitamin D animals reacted with redness and the control animals with a necrotic reaction. At the same time a BCG suspension was given intracutaneously. The vitamin D animals reacted with an ulcer and the controls with only a infiltrate. This does not seem to coincide with the earlier results. I believe that this is the phenomenon described by Sulzberger[6] as dichotomy, *i.e.* a strong reaction on the tuberculin and a weak reaction on a living but less virulent bacilli. The weak stimulus of the BCG caused a smaller reaction in the control animals who still reacted heavily on the human tubercle bacilli. The vitamin D-treated animals had not such a strong reaction on the human tubercle bacilli and therefore in this case reacted stronger to the second and weaker stimulus. The reaction on the tuberculin was the reverse of these reactions and coincided with the earlier results.

REFERENCES

1 L. v. D. Lugt and P. B. Rottier, *Acta Dermato-Venereol.*, 38 (1958) 264.
2 S. Lomholt, *Strahlentherapie*, 48 (1933) 655.
3 T. Jensen, *Ann. dermatol. syphilig.*, 36 (1948).
4 J. B. Ridderbos, *Ned. Tijdschr. Geneesk.*, 24 (1950) 228.
5 W. Klip, *Thesis*, University of Utrecht, 1951.
6 M. B. Sulzberger, *Dermatologic Allergy*, Thomas, Springfield, 1940.

DIE SONDERSTELLUNG DES LUPUS VULGARIS IN DER TUBERKULOSELEHRE

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Im allgemeinen trifft es für die Zonen gemässigten Klimas zu, dass die Erkrankungen an Lupus vulgaris zunehmen, wenn die Häufigkeit der allgemeinen tuberkulösen Erkrankungsfälle, insbesondere die Häufigkeit der Lungentuberkulose in der Bevölkerung wächst (Abb. 1). Nun gilt es seit dem Nachweis des Koch'schen Bazillus beim Lupus vulgaris (Doutrelepont) als eine befriedigende Erkenntnis, in eben diesem

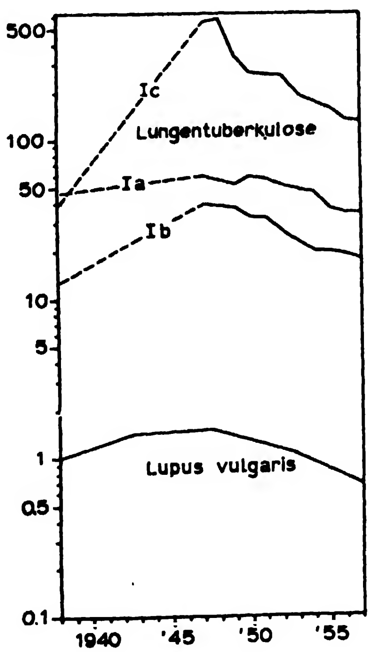

Fig. 1. Jährliche Neuerkrankungen an Lungentuberkulose und Lupus vulgaris auf 100,000 Einwohner Schleswig-Holsteins 1939–1957 (Zahlen für die Lungentuberkulose s. Lit.[10]).

Bazillus die wesentliche Ursache des Lupus vulgaris zu sehen; und man ist infolgedessen umso leichter versucht, auch aus der gleichsinnigen Beziehung zwischen der Häufigkeit der allgemeinen Tuberkulose und der des Lupus vulgaris zu folgern, dass die aus der allgemeinen Tuberkulose-Lehre abgeleiteten Vorstellungen über die

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Literatur S. 225

Pathogenese der tuberkulösen Erkrankung speziell auch die Natur des Lupus vulgaris dem Verständnis erschliessen würden.

Die erste Schwierigkeit für dieses Verständnis ergibt sich jedoch schon allein aus der Proportion der sehr voneinander abweichenden Häufigkeiten, mit der die verschiedenartigen Tuberkuloseformen auftreten. Wie die Abb. 1 erkennen lässt, verhalten sich in Schleswig-Holstein die jährlichen Neuerkrankungen an Lupus vulgaris zu den Neuerkrankungen an aktiven, ansteckenden — solche mit und ohne Bazillennachweis zusammengenommen — und nicht ansteckenden Formen der Lungentuberkulose ungefähr wie 1 : 75 und 1 : 250, wobei dieses Verhältnis anscheinend nicht einmal konstant ist. Es ist die Seltenheit, mit der es bei tuberkulöser Infektion zur Entstehung eines Lupus vulgaris kommt, die das Interesse für jene offenbar besonderen Umstände weckt, unter denen die Haut von einer lupösen Krankheitserscheinung befallen wird.

Über einige solcher Umstände täuscht man sich wohl nicht: der bevorzugte Befall des weiblichen Geschlechts und der bevorzugte Sitz der Erkrankung im Gesicht. Mag das erste Moment den Gedanken an die Bedeutung einer konstitutionellen Besonderheit fördern, so lenkt das zweite die Aufmerksamkeit auf eine topographisch umschriebene Veränderung des Gewebszustandes, auf den Zustand des Terrains also.

Zu einem wenig befriedigenden Ergebnis gelangt man dagegen, wenn man versucht, allgemeine Ideen der Infektionslehre wie die der Immunität oder die eines bestimmten Grades der Infektabwehr auf die Pathogenese des Lupus vulgaris zu übertragen. Es ist gewiss, dass die Träger einer Hauttuberkulose viel häufiger — vielleicht 10 mal so häufig (Gehrels und Kalkoff[1]) — als Hautgesunde von einer tuberkulösen Erkrankung der Lunge befallen sind. Bevorzugt trifft dies für die Männer zu. Verfolgt man dabei die Kasuistik im einzelnen, so ergibt sich, dass der Lupus vulgaris den Verlauf der Lungentuberkulose in gleichsinniger Weise begleiten, dass er aber auch abheilen kann, während die Lungentuberkulose fortschreitet, und dass schliesslich auch umgekehrt der Lungenbefund sich unter zunehmender Ausbreitung des Lupus vulgaris bessern kann. Die ergänzende Betrachtung der Todesursachen, unter denen das Leben der Lupösen endet, unterstreicht die Gefahr einer Lungentuberkulose im Verlaufe eines Lupus vulgaris. In Schleswig Holstein findet sich bei 448 in der Zeit von 1934 bis 1958 verstorbenen Lupus-Kranken als Todesursache 85 mal — 19% eine Tuberkulose der Atmungsorgane (57 Fälle) oder anderer Organe (28 Fälle) verzeichnet, wobei noch zu bemerken wäre, dass in 78 Fällen, = 17.4% des Gesamtmaterials, die Todesursache nur mangelhaft angegeben worden ist Freilich ist die Bedeutung einer solchen Mortalitätsstatistik nur schwer einzuschätzen, weil die Lupus-Kranken keineswegs in allen Fällen bis zum Lebensende verfolgt werden. In Schleswig Holstein ist es, wie vielfach auch anderwärts, üblich, die regelmässige Überwachung abzubrechen, wenn der klinische Befund nach Abheilung länger als 5 Jahre keinen Anhalt für das Wiederauftreten lupöser Infiltrate erkennen lässt; und dies ist bei den weitaus meisten Kranken der Fall. Gehrels und Kalkoff[1] haben daher guten Grund zu der Folgerung, dass der von der Mehrzahl der Untersucher gewonnene Eindruck eines verhältnismässig gutartigen Verlaufs der Lungentuberkulose bei Hauttuberkulösen sich durch die als Gegenargument dienenden Ziffern der Mortalitätsstatistik nicht widerlegen lässt.

Man kann nun aber beim Lupus vulgaris einerseits sehr rasch fortschreitende und andererseits über lange Zeiträume völlig unverändert bleibende Formen unterscheiden.

einer Dissertation über die Eigentümlichkeiten von Fällen mit solch extremen Verlaufsweisen hat Giefer[2] belegt, dass ein rasch progredienter Lupus häufiger als alle langsamer fortschreitenden Formen mit tuberkulösem Befall auch anderer Organe verbunden ist. Weder Geschlecht noch Lebensalter, noch familiäre Belastung, noch das ländliche oder städtische Milieu scheinen dabei eine Rolle zu spielen. Aber die Umstände der Flucht am Kriegsende, die Zeit des Weltkrieges 1914 1918 und der initiale Befall von Rumpf und Gliedmassen ist für den progredienten Verlauf offensichtlich von Belang. Ein der Dissertation von Weyer[3] über die Häufigkeit des Zusammentreffens lupöser Hauterkrankungen mit anderen tuberkulösen Manifestationen entnommenes Bild (Abb. 2) veranschaulicht den Unterschied in der allgemeinen

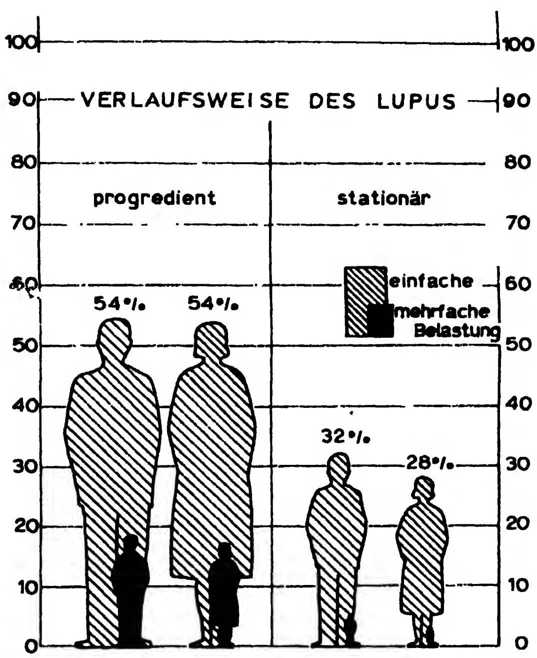

Fig. 2. Die Belastung mit Organtuberkulosen bei 1915 Fällen von Lupus vulgaris mit extremer Verlaufsweise.

tuberkulösen Belastung des Organismus bei rasch fortschreitendem Verlauf und bei stationärem Zustand des Lupus vulgaris. Aus diesem Befund lässt sich eher die These vertreten, dass die allgemeine Reaktionslage eines Organismus die Schnelligkeit in der Entwicklung und Ausdehnung des lupösen Vorganges bestimmt, als dass in ihr die Bedingungen, die die Entstehung eines Lupusherdes in die Wege leiten, enthalten sind.

Die Sonderstellung des Lupus vulgaris im Rahmen einer allgemeinen Tuberkuloselehre drängt sich auch aus den überzeugenden Erfolgen der Vitamin D Behandlung (Charpy) auf (vgl. 10. Internationaler Kongress der Dermatologie in London 1953: The treatment of tuberculosis of the skin). Es ist sicher, dass das Vitamin D keinen hemmenden oder gar zerstörenden Einfluss auf die Koch'schen Bazillen ausübt. Bei anderen tuberkulösen Manifestationen, insbesondere bei der Lungentuberkulose, ist es von nur sehr zweifelhaftem Wert. Wenn wir auch glauben, dass die modernen Tuberkulostatica aus vielerlei Gründen in der Behandlung des Lupus vulgaris gegen-

über dem Vitamin D einen weiteren Fortschritt darstellen (siehe bei Wagner[4,5]), so ist es für die hier versuchte Betrachtung dennoch wesentlich, dass die neue Ära der Lupusbehandlung, die durch eine bequeme orale Medikation gekennzeichnet ist, mit der Einführung eines Wirkstoffes beginnt, der gegen den Lupus vulgaris recht viel, gegen die übrigen tuberkulösen Manifestationen fast nichts vermag. Aus einem dem Morbus „Tuberkulosis" eigentümlichen Moment lässt sich der Einfluss des Vitamin D auf die lupösen Herde nicht begreifen. Hier ist die Bedeutung einer topographisch umschriebenen Veränderung des Gewebszustandes für die Pathogenese des Lupus vulgaris offensichtlich. Man kann nicht umhin, an dieser Stelle der Einführung der Lichtbehandlung durch Finsen zu gedenken. Der durch sie in der Lupusbehandlung bewirkte Fortschritt gegenüber der damals weit verbreiteten Exkochleation ist — auch im Hinblick auf das Verständnis für die Pathogenese des Lupus vulgaris — unvergleichlich tiefgreifender gewesen als der, der im Vergleich zur Vitamin D-Behandlung neuerdings durch die Tuberkulostatica erreicht worden ist. Man ersieht dies am besten aus den Berichten (z.B. Bering[6]) über die Pilgerfahrten, die seinerzeit die schleswig-holsteinischen Lupuskranken nach Kopenhagen unternahmen, bis v. Düring-Pascha, der erste Inhaber des Kieler Lehrstuhles für Hautkrankheiten, in Kiel selbst eine Finsenlampe in Betrieb genommen hatte (Proppe[7]).

Die grösste Schwierigkeit, die Pathogenese des Lupus vulgaris aus der allgemeinen Tuberkuloselehre abzuleiten, erwächst schliesslich aus der Altersverteilung des Erkrankungsbeginns. Man fasst den Lupus vulgaris gewöhnlich als eine Folgeerscheinung des Generalisationsstadiums auf. Wenn dies richtig sein soll, dann müsste der Beginn lupöser Herde eine zeitliche Beziehung zum Generalisationsstadium auf-

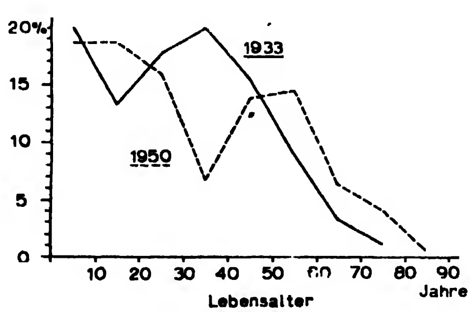

Fig. 3. Die Verteilung des Erkrankungsalters der in Schleswig-Holstein erfassten Lupuskranken in den Stichjahren 1931–1935 und 1948–1952.

weisen. Solange man den Eindruck als gesichert glaubte, dass der Lupus vulgaris weitaus am häufigsten die jugendlichen Altersklassen befalle, bestanden keine Schwierigkeiten für diese Vorstellung. 1953 konnten wir[8] aber darauf hinweisen, dass der Eindruck von einer Disposition des jugendlichen Alters zur lupösen Erkrankung auf einem Irrtum beruht. Immer wenn man bei einer so chronisch verlaufenden Krankheit wie dem Lupus vulgaris das Lebensalter des Erkrankungsbeginns in statistischer Weise betrachtet, ohne darauf zu achten, ob die Chancen der Erfassung für alle Lebensalter gleich gross sind, läuft man Gefahr, im jugendlichen Alter entstandene Krankheitsfälle bevorzugt aufzusummieren, weil die Krankheitsträger bei der spä-

ten Sammlung noch lebend angetroffen werden können, während die derselben Epoche zugehörigen, gleichzeitig ebenso erkrankten alten Leute sich entsprechend der Absterbeordnung der statistischen Erfassung inzwischen längst entzogen haben. In der Tat ergibt die Vermeidung dieses Fehlers, dass der Lupus vulgaris alle Lebensalter auswahllos ungefähr gleich häufig befällt.

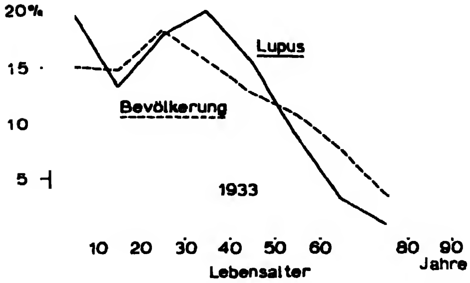

Fig. 4. Die Altersverteilung bei Lupusbeginn im Vergleich zum Altersaufbau der Bevölkerung Schleswig Holsteins in den Stichjahren 1931–1935.

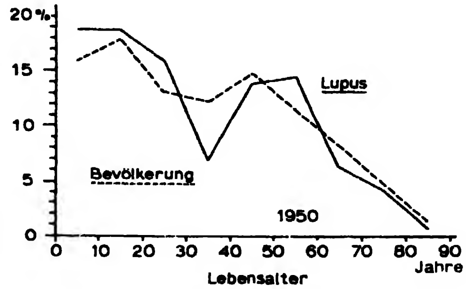

Fig 5. Die Altersverteilung bei Lupusbeginn im Vergleich zum Altersaufbau der Bevölkerung Schleswig-Holsteins in den Stichjahren 1948 1952.

Der Bedeutung dieses Argumentes wegen haben wir uns bemüht, dessen Stichhaltigkeit erneut zu prüfen. Aus ihm leitet sich die Erwartung ab, dass die Altersverteilung bei Lupusbeginn sich in den 30iger Jahren anders als in den 50iger Jahren darstelle, weil sich inzwischen der Altersaufbau der Bevölkerung geändert hat. Unsere Erwartungen[9] sind anhand der Karteiunterlagen des Lupusbeauftragten für Schleswig-Holstein (Prof. Dr. med. Albin Proppe) voll erfüllt worden. Die Abb. 3 zeigt, dass die Altersverteilungen des Erkrankungsbeginns in den betrachteten Stichjahren 1931/35 und 1948/52 verschieden ausgefallen sind. Die Abbildungen 4 und 5 demonstrieren die jeweilige Übereinstimmung mit der zugehörigen Altersverteilung der Bevölkerung. Eine Beziehung der Lupusentstehung zum Generalisationsstadium der tuberkulösen Infektion ist damit nur noch schwer vorstellbar.

LITERATUR

[1] P. E. Gehrels und K. W. Kalkoff, *Zbl. Haut-u. Geschlechtskrankh.*, 81 (1952) 121.
[2] W. Giefer, *Eigentümlichkeiten besonders progredient und besonders stationär verlaufender Lupusherde*, Inaug.-Diss., Düsseldorf, 1952.
[3] J. Weyer, *Die Häufigkeit des Zusammentreffens lupöser Hauterkrankungen mit anderen tuberkulösen Manifestationen*, Inaug.-Diss., Düsseldorf, 1955.
[4] G. Wagner, *Hautarzt*, 7 (1956) 126.
[5] G. Wagner, *Z. Haut-u. Geschlechtskrankh.*, 27 (1959) 161.
[6] F. Bering, *Münch. med. Wochschr.*, 52 (1905) 753.
[7] A. Proppe, *Hautarzt*, 8 (1957) 333.
[8] A. Proppe und G. Wagner, *Z. Haut-u. Geschlechtskrankh.*, 14 (1953) 376.
[9] A. Proppe und G. Wagner, *Z. Haut-u. Geschlechtskrankh.* 21 (1956) 1.
[10] *Tuberkulose-Jahrbücher, 1950–1957*, Springer, Berlin.

SOME EXPERIENCE WITH DIETHYL DITHIOLISOPHTHALATE (ETISUL), A PERCUTANEOUS ANTITUBERCULOSIS AGENT

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A symposium on the treatment of lupus vulgaris in the Memorial Congress to Niels Finsen indicates the importance of his contribution to the treatment of this disease.

In England, during the last 15 years the approach has become increasingly a chemotherapeutic one. Dermatologists there have now had the opportunity to assess the successes and practical disadvantages of such agents as calciferol, isonicotinic acid hydrazide and *p*-aminosalicylic acid.

Today I wish to record our experience with a new chemical which is administered percutaneously by inunction.

Our attention was drawn to diethyl dithiolisophthalate (Ditiphol, Etisul — I.C.I.) through its advent as a new weapon in Leprosy. Since the war with the increasing travel facilities, and at the same time the return of many British citizens consequent upon the granting of Independence to large areas of the Commonwealth, there has developed a small nucleus of cases of leprosy at certain clinics in the British Isles. Whilst discussing the treatment of one such case at the Manchester and Salford Hospital for Skin Diseases, Etisul or to give it its full name Diethyl dithiolisophthalate was mentioned, and it was remarked that much of the research work on the chemical had been carried out on tuberculous infections in animals. This is of course necessitated by the fact that it is impossible to provide a suitable infection with the bacillus leprae.

Very promising results had been seen with tuberculous infections particularly in mice (Davies and Driver[1]), where the effect of Etisul was comparable to that of isonicotinic acid hydrazide or streptomycin. It was less active than INH in the guinea pig (Davies and Driver[1]).

It would thus seem reasonable to apply these observations to the treatment of tuberculosis in man, particularly of the skin. Dr. Coburn and I therefore began a clinical trial in which cases of lupus vulgaris were treated by inunction of Etisul employing 5 g of the ointment twice weekly. This is rather a small dose, and our aim in later cases has been to increase it to 5 g daily. A preliminary report of our findings, and a brief account of the chemistry has been published in May of this year (Coburn and Marsden[2]). Today I would like to speak about our further experiences with Etisul.

We have commenced treatment in 17 cases, two of which have been excluded, and this report is therefore based on a total of 15 cases.

CASE NOTES

Case 1

F. Age 59 years. Attended since 1934 with lupus vulgaris of right and left cheeks. Local therapy; caustic agents, Kromayer lamp. Calciferol 9, 15, and 9 months courses. I.N.H. 5 years continuously. Condition before present treatment: Scars on both cheeks with a few active nodules. E.T.I.P.* 7 months. E.T.I.P. with I.N.H. 8 months. Result: Clearing of active nodules in scar.

Case 2

M. Age 75 years. Extensive lupus vulgaris of face and trunk of 65 years duration. Local therapy; caustic agents and X-rays 55 years ago. No further treatment until March 1959. I.N.H. 100 MGM. × 3 daily for 5 weeks with little change. E.T.I.P. 7 months with marked improvement. Now on I.N.H. whilst receiving plastic surgery for lymphoedema of the right cheek.

Case 3

M. Age 54 years. Attended since 1939 with lupus vulgaris of the nose, cheeks, and forehead. Local therapy; caustic agents, Kromayer lamp. Calciferol 18, 9, and 6 month courses. I.N.H. 24 and 21 month courses. Condition before present treatment: Still active nodules particularly on the forehead. E.T.I.P. 7 months. E.T.I.P. with I.N.H. 7 months. Result: Improvement. This patient complained of erythema after inunction when treated for 8 months, but patch tests were negative and treatment was continued without further trouble. The tip of the nose has been removed surgically for carcinomatous change in the scar.

Case 4

F. Age 41 years. Lupus vulgaris of right side of forehead of 5 years duration. No treatment. E.T.I.P. 10 months. Result: Considerable improvement particularly in the early stages. This patient also complained of erythema after inunction but continued treatment without any increase in this symptom. In May 1960 it was thought clinically that some new nodules had appeared in the right upper eyelid, and treatment with E.T.I.P. was discontinued. She has not been included in the cases showing benefit.

Case 5

F. Age 68 years. Attended since 1941 with lupus vulgaris of the right thigh. Local treatment; Kromayer lamp, X-ray therapy. Calciferol 4 and 6 month courses. I.N.H. and P.A.S. for 2 years continuously. Condition before present treatment: Still active nodules in scar. E.T.I.P. 8 months. Result: No benefit. Complained of erythema after inunction but patch tests negative.

Case 6

F. Age 58 years. Attended since 1934 with lupus vulgaris of the upper lip and right cheek. Local therapy; caustic agents and Kromayer lamp. Calciferol 24, 18, 6, 12, and 9 month courses. I.N.H. 5 years continuously. Condition before present treatment:

* E.T.I.P. = "Etisul" Imperial Chemical Industries, Pharmaceuticals Division, Manchester (England).

References p. 230

Scar with active nodules on right cheek. E.T.I.P. 10 months. Result: No benefit. In May 1960 new nodules were seen in the scar on the left cheek and treatment with E.T.I.P. discontinued.

Case 7

F. Age 45 years. Attended since 1946 with lupus vulgaris of the right thigh. Calciferol 15 months. I.N.H. 1 year, and 6 months courses. Condition before present treatment: Still active nodules in scar. E.T.I.P. 4 weeks, after which the patient objected strongly to the unpleasant smell of the treatment and refused to continue. Result: Refused treatment.

Case 8

F. Age 33 years. Lupus vulgaris of the forehead of 1 years duration. The only patient in the series with pulmonary tuberculosis which had been treated with Streptomycin, I.N.H. and P.A.S. and was now quiescent. E.T.I.P. 7 months. Result: Considerable improvement in the first 3–4 months, then little change. E.T.I.P. with I.N.H. 6 months. Result: Further improvement.

Case 9

F. Age 45 years. Attended irregularly since 1921 with lupus vulgaris of the nose and cheeks. Local therapy; Caustic agents, Kromayer lamp. Calciferol 24 months attended irregularly. I.N.H. 1 year — attended irregularly. Condition before present treatment: Scar with active nodules. E.T.I.P. started but patient defaulted and has not attended again. Result: Defaulted.

Case 10

F. Age 59 years. Attended since 1936 with lupus vulgaris of the right side of the neck. Local treatment; caustic agents, Kromayer lamp, X-rays. Calciferol 12 and 36 months. I.N.H. 9 and 4 months. Condition before present treatment: Scar with active nodules. E.T.I.P. 2 weeks following which an eczematous reaction developed in the treated area. Result: Secondary sensitization.

Case 11

F. Age 61 years. Lupus vulgaris of the left arm of 15 years duration. Local treatment; Kromayer lamp for 5 years with improvement, but relapsed 8 years ago, and has had no treatment since. E.T.I.P. 9 months. Result: Improvement. Patient complained of erythema after inunction at one period, but continued without further trouble.

Case 12

M. Age 48 years. Lupus vulgaris of the right ankle of 40 years duration. No previous treatment. E.T.I.P. 2 weeks after which an eczematous reaction developed. Patch tests to ointment and pure E.T.I.P. positive.

Case 13

F. Age 71 years. Lupus vulgaris of the right cheek of over 28-years duration. No

treatment for over 20 years. E.T.I.P. 2 weeks after which an eczematous reaction developed. Patch tests to ointment and pure E.T.I.P. strongly positive. Result: Secondary sensitization.

Case 14

F. Age 38 years. Lupus vulgaris of the lobe of the right ear of 15 years duration. I.N.H. said to cause vomiting. E.T.I.P. 2 months. E.T.I.P. with I.N.H. 4 months. Result: No improvement.

Case 15

F. Age 47 years. Lupus vulgaris of the right side of the neck of 30 years duration. I.N.H. 6 months but defaulted. Condition before present treatment: Scar with many active nodules. E.T.I.P. with I.N.H. 3 months. Result: No improvement.

Case 16

F. Age 67 years. Attended since 1947 with lupus vulgaris of the left ear. Calciferol 15 and 9 month courses. I.N.H. 2 months — defaulted. Condition before present treatment: No treatment for 5 years. Still active nodules lower margin of the left ear. E.T.I.P. with I.N.H. 2 months. Result: Some improvement.

Case 17

F. Age 57 years. Lupus vulgaris of the right side of the neck of 43 years duration. No treatment for 12 years. E.T.I.P. with I.N.H. 2 months. Result: Some improvement.

HISTOLOGY

The microphotographs are from Case 2, the biopsies being taken from adjacent areas on the back. Before treatment under the low power shows a well marked tuberculoid granuloma with giant cells. After treatment the whole picture is much less active, and the giant cells are few in number, and not clearly defined.

Under high power this alteration in the giant cells is well seen, and also a foamy appearance in the cytoplasm. This can also be seen in some of the epithelioid cells.

COMMENT

One case (9) defaulted and another 7 refused to continue treatment. These cases have been excluded.

I should mention here that although Etisul has a most unpleasant smell, the effect of this can be mitigated by care in the method of inunction. This should be carried out for 10 or 20 min, after which time the active principle will have penetrated the epidermis, and the residue of magnesium stearate and perfume can then be washed off. There will be a smell somewhat similar to garlic in the expired breath, due to the excretion of ethyl mercaptan, but this is not offensive to the patient, and if a suitable time of the day is chosen need not offend others since it only persists for about an hour.

The greatest care must be taken to avoid soiling the clothing. Most complaints are about the smell when this is laundered. It is due to the action of soap on Etisul with the formation of a most unpleasant compound. It can be prevented by washing the clothing in a non-ionic detergent.

References p. 230

Of the 15 cases remaining in the trial 5 (Cases 1, 2, 3, 4, and 8) showed improvement with Etisul alone for periods of 3 months or over. In case 4 however after 10 months treatment new nodules appeared and treatment had to be discontinued.

Spread of the lupus was also seen in Case 6 after 10 months inunction. This case had shown no clinical improvement. Resistance to Etisul after a period of treatment has been described in Leprosy (Davey[3]) and it would seem probable that a similar state of affairs could occur in tuberculosis. To reduce the risk of this, combined therapy as carried out in tuberculosis of other organs would seem the most reasonable approach. Coburn and I had in fact suggested this in our preliminary Communication.

Four cases have now been treated with combined therapy, Etisul by inunction and isonicotinic acid hydrazide by mouth. Three have shown improvement (Cases 11, 16, and 17) although the period of treatment is very short in the last two cases (4 and 6 weeks). One case (15) has shown no improvement after 7 weeks inunction.

Thus the results so far show improvement in 7 cases and no improvement in 3, spread of the lupus having occurred in 2 cases after 10 months treatment.

Toxic effects

Apart from the unpleasant smell, erythema following inunction is a not uncommon finding. It was a cause of complaint in four cases, and others admitted to noticing it when questioned. In one of these patients patch tests were carried out with negative results. All continued treatment without untoward effect.

In three cases however an eczematous reaction occurred (10, 12, and 13), in each case 2 to 3 weeks after commencing treatment. This was discontinued at once, and patch tests showed a sensitization to Etisul in the two cases in which it was carried out.

CONCLUSIONS

This is a short account of our clinical impressions of a new chemical with an action against the tubercle bacillus. In new cases it would seem advisable to combine its use with isonicotinic acid hydrazide by mouth. I think one of its most interesting possibilities lies in the effect on nodules fixed in the scar in cases which have received previous chemotherapy. The rather high incidence of sensitization in the white skin races is disappointing.

REFERENCES

[1] G. E. Davies, and G. W. Driver, *Brit. J. Pharmacol.*, 12 (1957) 434; 15 (1960) 122
[2] J. G. Coburn and C. W. Marsden, *Brit. J. Dermatl.*, 72 (1960) 192.
[3] T. F. Davey, *Leprosy Rev.*, 30 (1959) 61.

THE EPIDEMIOLOGY AND THE NATURAL HISTORY OF LUPUS VULGARIS CUTIS IN DENMARK 1895-1954*

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Lupus vulgaris of the skin is a tuberculous disease of world-wide occurrence. Precise accounts of the morbidity of the disease have so far been lacking. In many countries, however, the incidence and prevalence of active cases may be regarded as considerable. For this reason, and in view of the fact that the tuberculous infection often runs a serious course in patients with lupus, the disease is of importance in the fight against tuberculosis.

During the years 1895–1954 a total of 3,902 patients with lupus vulgaris of the skin was admitted to the Finsen Institute, Copenhagen, Denmark. In contrast to other series published on this disease, the present material was found adequate for an elucidation of the epidemiology of the disease, as well as for the natural history of and the clinical characteristics in the disease. The reason for this is that the material satisfies a number of essential requirements: *e.g.*, that the diagnosis was certain, that the material was representative of lupus for all Denmark, and that it was possible to carry out extensive comparisons with the general population. It was of particular importance that a comparison with the epidemiology of other forms of tuberculosis has been possible throughout the entire period.

The main results of the study have been the following:

Generally speaking, the incidence of lupus followed that of other forms of tuberculosis, both in time trends, geographic distributions and age patterns. The incidence in women was 2.4 times that in men. The disease was caused by both the human and bovine types of tubercle bacillus. Most often the human type was demonstrated; however an exact evaluation of the significance of the bovine tuberculosis for the lupus morbidity could not be made from the present material.

When lupus was the sole tuberculous disease, the mortality among the patients corresponded to that of the general population, but if another tuberculous disease was present – which was the case in 75% of the patients – the mortality increased. It was much higher with the occurrence of respiratory tuberculosis, which was present in 20% of the patients. Tuberculous diseases were a far more frequent cause of death among the patients than among the general population. The incidence of other forms of tuberculous disease was high also among lupus patients who did not die. Often, several organs were affected, or there were multiple affections within the same organ

* This study was supported by a grant from the Aage Bangs Fond, Denmark.

** Head: Poul V. Marcussen, M.D.

References p. 232

system An interesting observation was that other tuberculous diseases were frequent also among those in whom lupus was the first tuberculous manifestation.

The incidence of other tuberculous diseases was high among persons who had or had had lupus. This is of practical significance for the treatment of the patients and in the tuberculosis eradication programmes, where it is important to know which groups carry a higher risk than the general population. The high incidence mentioned also shows that lupus patients are not protected, "immunized", against other forms of tuberculous disease.

REFERENCES

1 O. Horwitz, *Acta Tuberc. Scand., Suppl.*, 47 (1959) 175.
2 O. Horwitz, *Lupus Vulgaris Cutis in Denmark 1895–1954*. The Finsen Institute, Copenhagen, 1960, 142 pp., 76 tables. Also published as *Acta Tuberc. Scand., Suppl.*, 49 (1960).
3 K. Clasen and O. Horwitz, *Advances Tuberc. Research*, 10 (1960) 237.
4 O. Horwitz and S. Christensen, *Am. Rev. Resp. Diseases*, 82 (1960) 862.

LUPUS VULGARIS

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The total outcome of treatment of *lupus vulgaris* with calciferol is given in Table I.

TABLE I

RESULT AFTER 10-YEARS OBSERVATION OF 210 PATIENTS SYMPTOM FREE AFTER TREATMENT WITH CALCIFEROL

Relapses	119	56.7%
Dead	15	7.1%
Symptom-free	76	36.2%

The patients have been under close permanent control and the symptom-free period has been registered. After 5 years observation 94% of the total of relapses in the period of ten years had occurred. 5 years has been used as the observation time in the following experiments. The results of treatment with increasing doses of INH are given in Table II.

TABLE II

TREATMENT WITH INCREASING DOSES OF INH

INH g	*No. of patients*	*No effect*	*Relaps*	*Symptom free after 5 years observation*
18	55	30 (54.5%)	16 (29.1%)	9 (16.4%)
30	63	7 (11.1%)	22 (34.9%)	34 (54%)
60	74	5 (6.8%)	16 (21.6%)	53 (71.6%)
90	17	1 (5.8%)	2 (11.6%)	14 (82.6%)
120	34	2 (5.3%)	0	32 (94.7%)

A total dose of 120 gram INH was sufficient to make 94.7% of the patients symptom-free over a period of 5 years. The addition of PAS had no influence on the final

outcome (Table III) but PAS is now used in the routine treatment to prevent bacterial resistance of *tubercle* bacilli in internal organs.

TABLE III

INFLUENCE OF ADDITION OF PAS

INH total dose	*INH 300 mg a day*		*INH 300 mg a day and PAS 14 g a day*	
	cure		*cure*	
g	+	−	+	−
60	27	10	26	11
120	22	2	10	0
	49	12	36	11
	61		47	

Streptomycin has not been used, partly for theoretical reasons, partly because of the age of the treated group. The outcome of the treatment of 166 consecutive cases is given in Table IV.

TABLE IV

OUTCOME OF TREATMENT OF 166 CASES

Symptom-free after 5 years of observation	144
Symptom-free but observed less than 5 years	20
Resistant	2

Chemotherapy in the cited doses is thus superior to earlier methods. The Finsen treatment will be used in the few cases which become resistant to INH—2 such cases have been noted.

Symposium 5

INITIAL MECHANISMS INVOLVED IN RADIATION EFFECTS

Chairman: B. LATARJET, Paris, France

Secretary: AXEL MADSEN, Copenhagen, Denmark

SOME DISTINGUISHING FEATURES OF THE INITIATION OF BIOLOGICAL DAMAGE BY PHOTON AND IONISING RADIATION

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I speak with diffidence, since I am not a photobiologist but a radiobiologist eager to learn from other participants about the response of living cells to visible and ultraviolet light. I have a number of questions to ask; and I shall indulge in a little speculation, in the hope that it will draw comments from those better informed than I am.

Let us consider first some of the similarities between the response of cells to ionising radiation and to photon radiation.

Ionising radiation is rather unspecific and unselective in its effects on molecules of all kinds, and we have to recognise at the outset that many characteristic features of the response of living cells to ionising radiations are an expression of the way cells are organised; in particular, of the uniqueness of the DNA-protein molecules which compose the genome, and possible uniqueness of certain RNA-protein molecules involved in gene reproduction. If the same small proportion of the molecules of each class is destroyed indiscriminately, the loss of those which occur in high multiplicity will not be noticed, and the observed response will be traceable to those which are unique. Thus, it comes about that, as a result of exposure to ionising radiation, not only gene mutation, but loss of infectivity in viruses and micro-organisms, and loss of proliferative capacity in cells of higher plants and animals, frequently exhibit dose-response relations which indicate that the effect is traceable to a chemical change initiated by one or by two ionising particles.

Photons, unlike ionising radiation, are selectively absorbed in certain molecular configurations. By choosing those photon energies in the U.V. which are strongly absorbed in the uniquely important nucleoprotein molecules — or by adsorbing on to the nucleic acid component of these molecules a dye such as acridine orange, and exposing the cell to photons selectively absorbed by the dye — a large proportion of the photon energy may be canalised on to the nucleoprotein. When this is done dose-response curves are observed for mutation, and for loss of reproductive integrity, which resemble those for ionising radiation, and indicate that the response is initiated by the absorption of 1, 2, or at most a few photons.

In order to discover the nature of the initiating events in any given case, we have to observe the manner in which the physical and chemical environment of the cell at the time of irradiation modifies the biological response, and compare these observations with those made on inanimate systems which are more amenable to chemical analysis. Here again, however, the organisation of the living cell, with its inherent capacity for "recovery" — either by repair or substitution — even in relation to gene mutation

References p. 242

— makes itself evident. This is true of both radiobiological and photobiological damage.

In radiobiology we have been able, for some years, to recognise two distinguishable ways in which environment during, or immediately following irradiation may modify biological response. One of these is essentially chemical and closely follows inanimate systems, while the other involves cellular metabolism. No doubt both classes of environmental influence exist also in photobiology, but the metabolic is the more obvious; indeed, comparable post-irradiation treatments, *e.g.* inhibition of protein synthesis, seem generally to reduce genetic damage induced by U.V. light more drastically than they reduce the same damage induced by ionising radiation.

The brilliant work of Witkin[1], four years ago initiated a train of investigations which appear to have established three stages in ultraviolet mutagenesis:

(*1*) A chemical change (promutation) resulting directly from the absorption of the U.V. photon.

(*2*) Stabilisation of this change against decay, by a process dependent on protein synthesis and hence on the availability of amino acids.

(*3*) Further metabolic steps whereby the promutation becomes converted into a permanent functional mutant entity in the genome.

Doudney and Haas[2–5] have concluded that the initial change is not in the material of the genome itself, but in an RNA precursor, present in the cell at the time of irradiation. This interpretation still awaits confirmation.

Our knowledge of the initiation of mutation by ionising radiation is advancing along similar lines, but is, as yet, more hazy than the picture which I have just presented of mutation induction by ultraviolet light.

Having in mind these ways in which biological organisation imposes certain common features on the response of cells to all types of radiation, we may consider some of the characteristic differences between damage initiated by the different types of radiation — divided broadly into "ionising radiation", "ultraviolet light" and "visible light".

It is of interest first to compare the two extremes — visible light and ionising radiation.

Red light used in photosynthesis transfers to the receptor molecules about 1.8 eV in a single act of absorption.

The visual sensation of green light starts with the transfer of 2.5 eV, in a single act, to the retina. As against this, a single energy transfer from a fast ionising particle to a macromolecule is most frequently around 100 eV.

At first glance one is inclined to say that the low energy quanta are physiologically useful to the cell (indeed, essential to life) whereas the larger quanta are inherently damaging — with the dividing line set at about 4 eV (*i.e.* in the U.V.) Closer examination of the facts, however, reveals that the distinction between constructive and destructive effects is not simply a matter of quantum energy. On the one hand ionising radiation can give rise to ordinary physiological stimuli. For example, a dose of less than 1 R has been observed by Lipetz[6] to give a perceptible signal on an electroretinogram of the frog, and a minute X-ray dose of 1 mR given in a single flash is reported to produce the sensation of vision in the human dark adapted eye[7]. Another example is provided by the water beetle *Daphnia magna* which changes its swimming habit in response to a flash of X-rays — an effect which was traced by Baylor and Smith[8] to the stimulation of the nauplius eye of this animal.

Hug and others have recently become much interested in this branch of radiobiology and have added many interesting examples of physiological stimulation[9].

On the other hand, visible light may be very damaging — witness the many well known examples of photodynamic action, and the recent work of Kihlman[10,11] on the induction of chromosome structural damage by visible light (2.3–2.8 eV) acting on root tip cells in which acridine orange had been adsorbed onto the nucleic acids. All the well known types of chromosome structural damage are produced, including chromatid breaks, isolocus breaks and exchanges.

I do not find it too surprising that ionising radiation should be able to stimulate the retina of man and frog, and the nauplius eye of *Daphnia*. This latter effect can also be brought about by reducing dyes, and it is well known that, through the decomposition of water, ionising radiation can reversibly reduce dyes such as methylene blue[12]. The retina, in fact, could present an ideal radiation detector, since the optic nerve is stimulated by an excitation or chemical change in a very small number out of a large array of identical pigment molecules. If the excitation is one that can be produced by ionising radiation, then stimulation should occur (accompanied by changes of a destructive nature in other pigment molecules, which go undetected) when the retina is exposed to quite low doses.

I find the damaging effect of visible light — especially the production of chromosome structural damage — more interesting. Cells habitually exposed to visible light have evolved a means of protecting themselves against this potentially damaging agent, or even harnessing the energy. The chloroplast and the retina are evidently very elaborate devices. There may be other simpler ones. It has, for example, been suggested that pigmented bacteria are protected by an intimate association between the pigment molecules and caratenoids or other conjugated double bond structures, which drain away the energy harmlessly. Radiobiology has surely much to learn from these branches of photobiology.

The remainder of my remarks will be concerned with the effects of ultraviolet light, compared on the one hand with those of visible light, and on the other with those of ionising radiation.

From the standpoint of interpretation, studies with ultraviolet light offer the great advantage over those with ionising radiation that by means of "action spectra" it is possible, for any given type of biological response, to specify the kind of molecule in which the significant energy is primarily absorbed. By this test some forms of U.V. damage are seen to arise mainly from energy absorbed in protein, and others from energy absorbed in RNA, DNA, or nucleotides. We shall consider the latter forms of damage which include mutation, chromosome structural damage, and loss of reproductive integrity in viruses, bacteria and the proliferative cells of higher plants and animals.

Initiation of these forms of damage by U.V. and ionising radiation differs outstandingly in two respects:

(*1*) As induced by ultraviolet light they are, in many instances, partially restorable by subsequent exposure to visible light. As far as I know, this has never been observed when the same forms of damage are initiated by ionising radiation.

Photorestoration of U.V. damage, which has been excellently reviewed by Jagger[13], will, no doubt, be fully treated by other speakers. I wish only to remark on one fac

References p. 242

which interests and puzzles me very much, namely that I have never found in the literature any reference to photorestoration of chromosome structural damage induced by ultraviolet light, although this shows a "nucleic acid" type of action spectrum. Kirby-Smith and Craig[14] looked for it in irradiated dry pollen of *Tradescantia*, and failed to find it. I would be very glad to know of other experimental data on this point.

(2) The second difference concerns the participation of oxygen in the act of initiation which is so characteristic of ionising radiation. The forms of damage which we are considering are apparently induced by ultraviolet light to the same extent, whether oxygen is present or absent at the time of irradiation. This is indicated by a minus sign in the Table in the position corresponding to biological damage induced by U.V. It will be seen that chemical reaction induced by all forms of radiation (ionising, U.V. and visible) are frequently oxygen sensitive. Quite recently Alexander and Moroson[15] reported that changes in dilute solutions of salmon sperm DNA, as judged both by intrinsic viscosity and average molecular weight, were strongly oxygen dependent when induced by ultraviolet light.

The lack of oxygen dependence for biological damage induced by ultraviolet light surprises me, not only because many photochemical reactions are greatly influenced by the presence of oxygen, but also because many authors believe that peroxides are intermediates (in some cases at least) in the induction of mutation by ultraviolet light.

All forms of damage under consideration, including some 8 or 9 reverse mutations, are known to be oxygen sensitive when induced by ionising radiation. One of these mutations is to purine independence[16] in *E. coli* B/r the others are reversed mutations to growth factor independence in yeast[17]. The apparent absence of oxygen dependence for damage induced by ultraviolet light surprised me so much that I asked my colleagues Dr. Dewey and Dr. Boag to carry out some experiments on the U.V. inactivation of bacteria (*Serratia marcescens*) in the presence of oxygen at 100 atmospheres. The experiments are not complete, but no oxygen effect has been observed, and the indications are that none exists at this pressure.

TABLE I

EFFECT OF OXYGEN ON CHEMICAL AND BIOLOGICAL RESPONSE TO RADIATION

Chemical response	*Type of radiation*	*Biological response*
− (10 mmoles/l)[a] + (400 mmoles/l)[a] (680 atm)	α-radiation (5 ÅU/100 eV)[b]	(1 mmoles/l)
+	Proton radiation (50 ÅU/100 eV)[b]	+
+	X and γ-radiation (Up to 5000 ÅU/100 eV)[b]	+ (4 μmoles/l)
+	Ultraviolet light	−
+	Visible light	Photodynamic + Acridine orange +

[a] Concentration of dissolved oxygen.
[b] Path length for dissipation of 100 eV.

In trying oxygen at 100 atmospheres I had in mind the possibility that the biologically important intermediates formed by ultraviolet light might, for one reason or another, have much shorter lifetimes than those formed by ionising radiation. The pressure at which oxygen can participate is inversely related to the lifetime of the species with which it reacts. This is well illustrated by the top line of Table I. H-atoms are formed very close together along the track of an α-particle in water, and on this account would be expected to have a very short lifetime. Correspondingly, an influence of oxygen on a chemical reaction induced by α-radiation was not observed till quite recently — and then only by the use of oxygen at 940 atmospheres[18]. Biological effects of α-radiation appear not to have been tested at oxygen pressures above 1 atmosphere.

Evidently those intermediates formed by ultraviolet light and α-radiation which give rise to biologically important reactions, either do not require oxygen to become toxic, or are of such short lifetime that oxygen cannot compete at the pressures so far tested.

The "non-conformity" of ultraviolet light remains something of a mystery (to me) and I should like to conclude with a little speculation which might serve to bring these two remarkable features of the initiation of biological damage by ultraviolet light into relation with three other experimental observations.

I. That when biological effects which show a "nucleic acid" type of action spectrum are considered, and damage induced by ultraviolet light photons of maximum efficiency is compared with that induced by ionising radiation on the basis of energy absorbed per g of nucleic acid, then broadly speaking ultraviolet light is:

(*a*) Considerably less efficient than ionising radiation in inactivating transforming principle[19], and inactivating bacteriophages[19,20] T2, S13 and ΦX174, in inducing lysogeny in *E. coli* K12 (λ)[21] and in inducing mutations in microorganisms.

(*b*) Very much less efficient in destroying the reproductive integrity of mammalian cells[20] (dose ratio 20,000 : 1).

(*c*) Extremely inefficient in producing chromosome structural damage[14,22-24] (dose ratio 2 million : 1).

These statements are to be understood in a qualitative rather than a strictly quantitative sense, especially that regarding mutagenesis. As far as I am aware mutation induction in phage and bacteria has always been observed to vary as the first power of the dose in the case of ionising radiation, and as a higher power of the dose (at low doses) with ultraviolet light. At higher U.V. doses a "saturation" effect usually sets in. No single figure for the relative biological efficiency of the two radiations can therefore be given. However, the fact that at the dose levels of U.V. and ionising radiation commonly employed, a given yield of mutations per survivor is obtained when the U.V. dose (ergs/g) to nucleic acid is ten times the X-ray dose, contrasts strikingly with the ratio of 20,000 : 1 for loss of reproductive integrity by HeLa cells (where the kinetics of inactivation are essentially the same for both radiations) and about 2 million : 1 for the production of breaks in *Tradescantia* chromosomes.

II. The second is that when intact tobacco mosaic virus is inactivated by U.V. it is found not to be photoreactivable; but when the infective RNA of tobacco virus is inactivated by U.V. it is photoreactivable[25].

III. The third is an exception to the general rule that the effects of ionising radia-

References p. 242

tion are oxygen dependent. Almost pure DNA in the form of transforming principle, and extracellular phage, exposed to ionising radiation do not show oxygen dependence except in the presence of high concentrations of —SH compounds (or cell debris).

My speculation is (*1*) that U.V. energy absorbed in simple nucleic acids or nucleotides is biologically efficient and photoreactivable; and (*2*) that U.V. energy absorbed in nucleoprotein is biologically inefficient (possibly because the energy of excitation is almost immediately transferred from the nucleic acid to the protein[25]) and not photoreactivable; and (*3*) that nucleoprotein damage is generally oxygen dependent, whereas nucleic acid alone, whether excited by ultraviolet light or ionising radiation, is only oxygen dependent in special circumstances, at least with respect to forms of chemical change which are important for its own subsequent duplication.

It is not hard to find apparent objections to this simple speculation, which should not perhaps be taken too seriously.

It may be noted, however, that

(*1*) It accords with the photoreactivation of ultraviolet light induced (pro) mutation if the Doudney and Haas hypothesis is correct.

(*2*) It accords with the efficient induction of chromosome structural damage by visible light absorbed and transmitted to DNA by acridine orange, since the DNA combined with acridine orange presumably cannot also be in combination with protein. This inference seems justified by observations such as those of Oster[26], who found that whereas the fluorescence of dyes such as acridine orange is strongly quenched by dilute solutions of DNA, it is not quenched by thymonucleohistone.

(*3*) It is not at variance with the photoreactivation of irradiated phage since the DNA of extracellular phage is presumably compact, and not in the form of nucleoprotein.

REFERENCES

1 E. M. Witkin, *Cold Spring Harbor Symposia Quant. Biol.*, 21 (1956) 123.
2 C. O. Doudney and F. L. Haas, *Proc. Natl. Acad. Sci. U.S.*, 44 (1958) 390.
3 C. O. Doudney and F. L. Haas, *Proc. Natl. Acad. Sci. U.S.*, 45 (1959) 709.
4 C. O. Doudney, *Nature*, 184 (1959) 189.
5 F. L. Haas and C. O. Doudney, *Proc. Natl. Acad. Sci. U.S.*, 43 (1957) 871
6 L. E. Lipetz, *Radiation Research*, 2 (1955) 306.
7 L. E. Lipetz, in A. A. Buzzati-Traverso (Editor), *Proc. Venice Symp. Radiation Biol.*, Taylor and Francis, London, 1960, p. 227.
8 E. R. Baylor and F. F. Smith, *Radiation Research*, 9 (1958) 406.
9 O. Hug, in A. A. Buzzati-Traverso (Editor), *Proc. Venice Symp. Radiation Biol.*, Taylor and Francis, London, 1960, p. 227.
10 B. A. Kihlman, *Nature*, 183 (1959) 976.
11 B. A. Kihlman, *Exptl. Cell Research*, 17 (1959) 590.
12 G. Stein, *Discussions Faraday Soc.*, 12 (1952) 227.
13 J. Jagger, *Bacteriol. Rev.*, 22 (1958) 99.
14 J. S. Kirby-Smith and D. L. Craig, *Genetics*, 42 (1957) 176.
15 P. Alexander and H. Moroson, *Nature*, 185 (1960) 678.
16 E. H. Anderson, *Proc. Natl. Acad. Sci. U.S.*, 37 (1951) 340.
17 R. L. Gunther, personal communication.
18 C. N. Trumbore and E. J. Hart, *J. Phys. Chem.*, 63 (1959) 867.
19 R. Latarjet, this conference.
20 H. H. Lee and T. T. Puck, *Radiation Research*, 12 (1960) 340.
21 H. Marcovich and R. Latarjet, *Advances Biol. and Med. Phys.*, 6 (1958) 75.
22 C. P. Swanson, *Proc. Natl. Acad. Sci. U.S.*, 26 (1940) 366.
23 C. P. Swanson, *Genetics*, 27 (1942) 491.
24 C. P. Swanson, *J. Gen. Physiol.*, 26 (1943) 485.
25 F. C. Bawden and A. Kleczkowski, *Nature*, 183 (1959) 503.
26 G. Oster, *Trans. Faraday Soc.*, 47 (6) (1951) 660.

INDUCTION OF NUCLEAR DAMAGE BY IONIZING AND ULTRA-VIOLET RADIATION

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Radiation induces cell death by several different mechanisms. The manner in which death is induced and the syndromes associated with cell killing unquestionably change in type and frequency from organism to organism, from tissue to tissue, from cell to cell. Also, ionizing and ultraviolet radiation induce a spectrum of biological effects usually differing in frequency, occasionally overlapping, and undoubtedly differing at the lowest level of biological amplification, that is, at the point of energy absorption.

In assessing radiation effects on the nucleus we have used the haploid–diploid genetic system of the wasp *Habrobracon*, and corroborated and extended this work with the fruit fly *Drosophila*. The value of the haploid cell lies in its lack of two genetic complements which would buffer, cover, or alter the radiation effects. In *Habrobracon*, unfertilized eggs develop into normal haploid males, and fertilized eggs develop into females. The study of irradiated, unfertilized eggs can be combined with the study of irradiated eggs fertilized by unirradiated sperm to detect different kinds of nuclear damage. "Damage" is used here in the sense of induction of death of the cell either immediately after irradiation or after few or many mitoses have occurred.

MANIFESTATION OF NUCLEAR DAMAGE

Nuclear damage can be divided into classes according to the various syndromes associated with death of the cell or embryo. These are summarized in Table I. That dominant lethality*** is a complex of different radiation effects on the nucleus has been recognized by genetic means in *Habrobracon* (Atwood *et al.*[1]) and *Drosophila* (Parker[2]). Cytological criteria have been established which make possible a more accurate discrimination among classes of lethality (Von Borstel and Rekemeyer[3]; Whiting *et al.*[4]).

Type I

Type-I lethality is expressed as a sharp depression of the rate of mitosis, with cessation after a few divisions. This is the most frequent type of dominant lethality

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*** Dominant lethality is defined as nuclear damage of any extent that cannot be completely covered for at least one sexual generation by a normal nucleus.

References p. 249/250

TABLE I

A SUMMARY OF THE SYNDROMES AND PROBABLE DAMAGE SUSTAINED BY CELL NUCLEI FROM RADIATION
see text for a complete description

Nuclear Damage	*Syndromes*	*Basis*
Type I	Death after one or a few nuclear divisions; syndromes vary from organism to organism.	Inhibition of mitosis
Type II	Death after many nuclear divisions. In the wasp *Habrobracon*, characterized by death after blastoderm formation and before hatching in unfertilized eggs; death occurs predominantly after hatching in fertilized eggs.	Loss of parts of chromosomes
Type III	Death after many nuclear divisions. In *Habrobracon* death occurs after blastoderm formation and before hatching in unfertilized and fertilized eggs.	Extensive loss of chromosomes or chromosome parts.
Type IV	Death and pycnosis soon after irradiation — characteristic of certain cells only, *e.g.* lymphocytes, young primary gametocytes, late gonial cells, and neuroblasts. (Possibly nuclear damage, but not established.)	Unknown, but possibly inhibition of an energy pathway.
Type V	Dominant lethality expressed in eggs after irradiation in the gonial stage. In *Habrobracon*, embryo dies late in development. This type seems to be an enhancement of normal aging processes. (Possibly nuclear damage, but not established.)	Unknown, but possibly impairment of function of genes that operate during oocyte differentiation.

induced in eggs or sperm of *Habrobracon* or *Drosophila* by ionizing radiation (Von Borstel and Rekemeyer[3]; Von Borstel, unpublished), and in *Habrobracon* gametes, at least, by nitrogen mustard (Von Borstel[5]). The syndromes associated with this very early death differ between *Habrobracon* and *Drosophila* (Von Borstel[6]), and undoubtedly among other animals as well. For example, Brachet[7] has described an interesting lethal syndrome induced by nitrogen mustard in amphibian gametes, and Puck and Marcus[8] have described formation of giant cells in irradiated tissue cultures. In *Habrobracon*, the Type-I lethal syndrome is associated with production of feulgen-negative nuclei. In *Drosophila*, the early death syndrome is characterized by the presence of several polyploid, disturbed, basket-shaped "metaphases" with chromosomes lying at random in the spindle (Von Borstel[6,9]; Sonnenblick[10]).

It has been possible to demonstrate in *Habrobracon* that the Type-I lethal syndrome can be induced by agents that inhibit meiosis or mitosis (Von Borstel and Atwood, unpublished). Therefore, it can be concluded that depression of the rate of the mitoses following meiosis is a sufficient action of the radiation to bring about the Type-I lethality syndrome. Interestingly enough, the rate of meiosis is not depressed in *Habrobracon* by ionizing radiation.

It has been known for years that mitosis can be slowed by ionizing or ultraviolet radiation (*cf.* Carlson[11]); also, it has been shown that irradiated cells containing broken chromosomes divide much more slowly than irradiated cells that appear to have normal chromosomes (St. Amand[12]). That mitosis is indeed inhibited by formation and, presumably, breakage of chromosome bridges without attendant irradiation is demonstrable in *Drosophila*. By use of the tandem metacentric X-chromo-

ome of Novitski and Lindsley[13] where meiotic anaphase-II bridges are formed as a consequence of crossing over (Novitski[14]), it was possible to show that death occurs very early with the "basket metaphase" syndrome in the proportion of eggs expected to have a broken chromatid incorporated into the pronucleus (Von Borstel[6,9]).

In summary, it seems probable that Type-I dominant lethality induced by ionizing radiation results from inhibition of mitosis caused for the most part by induction of bridges.

Type II

Type-II lethality in unfertilized eggs is expressed as death after blastoderm formation and before hatching. In X-irradiated eggs fertilized with a normal sperm, Type-II lethality is primarily expressed as death of the embryo just prior to hatching and in the early larval stages after hatching although some do not die until the pupal stage of development. How Type-II lethality manifests itself can be seen in Fig. 1a. It is observed in eggs irradiated in the first metaphase of meiosis.

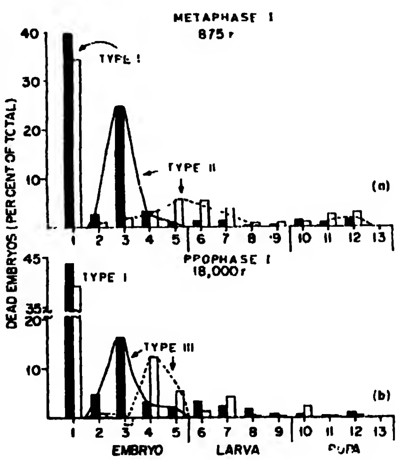

Fig. 1. Frequencies of death at different stages of development of *Habrobracon* embryos irradiated as oocytes in the first meiotic metaphase and prophase. Solid columns, unfertilized; open columns, fertilized with unirradiated sperm. Numbers on the abscissa designate stages of development. Lines connect the bars for ease in visualizing the fertilization-induced delay in expression of death, especially in eggs irradiated in the first meiotic metaphase (redrawn from Von Borstel and Rekemeyer[3]).

This type of lethality can be attributed to chromosome imbalance of limited extent, and it has been possible to mimic it by creating imbalance by means other than radiation (Von Borstel[6]; Von Borstel and Rekemeyer[3]). For instance, chromosomal translocations were induced and then the offspring of translocation heterozygotes were scored. Of such offspring (depending upon the individual translocation), approximately half have unbalanced chromosome complements consisting of a duplication in one chromosome arm and a deficiency in another. The duplication-deficiency products of 27 different translocations have exhibited syndromes identical to the Type-II lethality induced by radiation. Unfertilized, the embryos die after blastoderm formation and before hatching; fertilized with normal sperm, death occurs later, predominantly after hatching.

References p. 249/250

In summary, it seems likely that Type-II dominant lethality is caused by radiation induction of slight chromosomal imbalances in the haploid genetic complement.

Type III

Type-III lethality is expressed as death after blastoderm formation and before hatching in both unfertilized eggs and eggs fertilized after irradiation. How this lethality manifests itself can be seen in Fig. 1b. It is observed in eggs irradiated in the first prophase of meiosis.

Type-III lethality can be attributed to chromosomal imbalance of large extent. It has been possible to mimic Type-III lethality by construction of triploids. Most of the offspring of *Habrobracon* triploids can be considered to have extreme imbalance of their genetic complements. The normal haploid number of chromosomes is 10 and the diploid number is 20; the offspring of a triploid virgin would have a chromosomal number anywhere between 10 and 20 with the mode probably being around 15. Embryos chromosomally imbalanced to this degree usually die after blastoderm formation and before hatching. Fertilization with normal sperm still results in an abnormal chromosome complement, though more balanced, but still only a few percent survive to post hatching periods of development. Most of the inviable offspring from *Drosophila* triploids and translocation heterozygotes also die after blastoderm formation and before hatching (Von Borstel and Rekemeyer[3]).

In summary, it is likely that the Type-III dominant lethal syndrome induced by ionizing radiation in meiotic prophase-I is also caused by chromosome imbalance phenomena, though probably of larger extent than that induced in meiotic metaphase-I.

Type IV

Type-IV lethality appears to be associated with cells that are often alleged to be, and in some cases actually may be, the most sensitive to radiation of all types of cells. Killing, as characterized by pycnotic appearance of the cell nucleus, is rapid, and occurs among non-dividing cells. Thus, the manner of killing is such that mitotic inhibition would seem to be ruled out as the primary radiation effect Similarly, the chromosomal complement, even if broken or rearranged, remains balanced since nuclear divisions are needed to upset gene balance. Some of the cells which appear to be subject to Type-IV lethality are: in mammals, lymphocytes (Trowell[15]), Type-B spermatogonia (Oakberg[16]), neuroblast cells (Hicks[17]), and young primary oocytes (Oakberg[18]); in insects, young primary oocytes (Whiting[19]; Grosch and Sullivan[20]) and young ganglion cells (Gaulden, pers. comm.).

It has been suggested that the rapidity of death of these cells must be caused by inhibition of an immediate vital process, such as that of an energy pathway (L. S. Kelly, pers. comm.). The research of Creasy and Stocken[21] would indicate that such damage may indeed be primary, but their work needs to be repeated and extended. A good clue to the nature of Type-IV lethality is inherent in the "rejoining system" of Wolff and Luippold[22] which is sensitive to radiation. Wolff[23] has shown, at 600 R, that at least two hours elapse before broken chromosomes rejoin; when the cell is irradiated at the same dose and is in a state of chemically induced anoxia, rejoining occurs within one hour. This is consistent with the expectation that a dose-effect relation exists in the "rejoining system". Sobels[24] has shown, following post-irradiation treatment with hydrocyanic acid, that genic mutations occur at higher frequency with

high intensities of radiation than after low intensities, or than after either low or high intensities without posttreatment, indicating that Wolff's and Luippold's system is not restricted to broken chromosomes alone. It seems possible that the radiation-sensitive "rejoining system" could be the crucial element for cell viability in cells that exhibit the Type-IV lethality. Consistent with this explanation, Grosch[25] (and pers. comm.), to study Type-IV killing in young oocytes of *Habrobracon*, has used the sensitive fractionation procedure devised for *Vicia* chromosomes by Wolff and Atwood[26]. Grosch finds that young oocytes are subject to a small repair and oogonia are subject to a large repair when the dose is fractionated.

A study of the biochemical basis for Type-IV killing is needed to gain an understanding of a primary effect of radiation on a radiation-sensitive system that probably occurs in many cell types, but in certain cells is immediately essential for survival.

Type V

La Chance[27] demonstrated that the lowered hatchability observed in *Habrobracon* eggs treated in oogonial stages was a case of true dominant lethality; survival frequency was not increased following fertilization with normal sperm. Upon repetition of his experiments (Von Borstel, unpublished data) it was found that dominant lethality induced in oogonia by X-radiation is expressed during late embryogeny under conditions which indicate that the embryo is excessively fragile; the gut ruptures or herniates at the stage of development when peristaltic contractions of the gut begin. Interestingly enough, this syndrome is seen after ultraviolet irradiation of the cytoplasm of mature eggs (Amy and Von Borstel[28]; Von Borstel[29]). A complicating factor is that eggs from normal unirradiated females have a depressed hatchability as the females age. The Type-V syndrome is expressed in embryos from these aged females. Therefore, the Type-V lethality may not be nuclear but may be a systemic or even a cytoplasmic effect of the radiation.

Although the nature of this type of lethality is unknown, the results can be explained in a consistent manner if it is assumed that the radiation effect is upon the nucleus of the gonial cell. It is possible that radiation inactivates genes in the gonial cell which are not necessary for immediate survival but are needed to properly synthesize the cytoplasm when the gonial cell develops into a mature determinate egg. Other explanations involving systemic or cytoplasmic effects also may be invoked, but these tend not to be so easily reconciled with the data. A complete description of Type-V lethality and other effects observed after irradiation of the gonial system will be published elsewhere.

COMPARISON OF ULTRAVIOLET AND IONIZING RADIATION EFFECTS ON THE NUCLEUS

Several responses of cells to radiation show marked differences according to whether the source is ionizing radiation or ultraviolet radiation: Photoreactivation is a notable example of an effect capable of occurring to a marked degree only after ultraviolet irradiation. In general, it has been observed that chromosome breakage is less extensive after ultraviolet radiation than after ionizing radiation. Further, survival curves with ionizing radiation often indicate a lower multiplicity of targets or a lower threshold before the lethal action appears than does ultraviolet radiation. As an example of the last mentioned, response of newly laid *Habrobracon* egg hatchability

to α-radiation is exponential (Rogers and Von Borstel[30]) and to ultraviolet radiation the response is approximately eight-hit (estimated by Kimball's[31] method from data of Amy and Von Borstel[28]).

In *Habrobracon*, it has been observed that following treatment of oocytes with ionizing radiation a small consistent fraction of Type-I lethality is repaired when the egg is fertilized with a normal sperm. This is shown in Table II. Magni[32] has observed

TABLE II

FREQUENCY OF X-IRRADIATED EGGS DYING DURING EARLY DEVELOPMENT FROM DEATH ATTRIBUTED TO INHIBITION OF MITOSIS

calculated from data of Von Borstel and Rekemeyer[3]

Stage irradiated and dose (R)	*Type I/Total eggs*	
	Unfertilized	*Fertilized*
Metaphase I,		
875	0.399	0.341
1100	0.567	0.528
Prophase I,		
15000	0.440	0.421
18000	0.437	0.392

a similar repair, which could not be shown to be a genetic defect in the progeny, when X-irradiated haploid yeast were mated with unirradiated yeast. Atwood[33] has observed such a phenomenon in *Neurospora* with ultraviolet radiation where conidial cells are heterokaryotic, made up of several haploid nuclei. The effect with ultraviolet light is much more pronounced than that seen after X-irradiation (Atwood and Mukai[34]).

Therefore it seemed possible that the nuclear reactivation phenomenon could be amplified if ultraviolet radiation were to be used on *Habrobracon* eggs. Unfortunately, since the ultraviolet irradiations must be performed on newly laid eggs, it is not possible to fertilize the eggs after irradiation; eggs are fertilized during the process of oviposition. The consequent doubling of target size by fertilization is offset by several factors: The sperm nucleus does not combine with the egg nucleus until meiosis is completed and the egg nucleus is irradiated while it is undergoing meiosis; the sperm enters the egg at the wide end during oviposition, and, since the wide end of the egg is not directly irradiated for inactivation of the nucleus (see Von Borstel and Wolff[35], for irradiation procedures), the sperm may be less available as a target: even though the sperm may not be out of the range of the ultraviolet radiation, at doses below 50% survival the chance is low for inactivation of both the egg and sperm nucleus.

The results can be seen in Fig. 2 where only the early death data, presumed to be inhibition of mitosis, are recorded. Sufficient precautions were taken to prevent photoreactivation. It is obvious that at low doses the fertilized eggs showed a marked recovery, and that at high doses the fertilized eggs are more sensitive to the radiation. The theoretical curve for double the target size is not approximated by the fertilized eggs. As a further check, sex ratios of survivors were determined at the different doses of ultraviolet radiation. As would be expected from the shape of the curve for

the irradiated fertilized eggs, the percentage of females increased over the normal at the first two doses and decreased at the highest dose; androgenetic males were not observed. Nuclear reactivation is clearly observable after ultraviolet irradiation. Therefore, it seems likely that the Type-I lethality from irradiation of the nucleus can be induced through at least two distinct mechanisms, one capable of being reactivated.

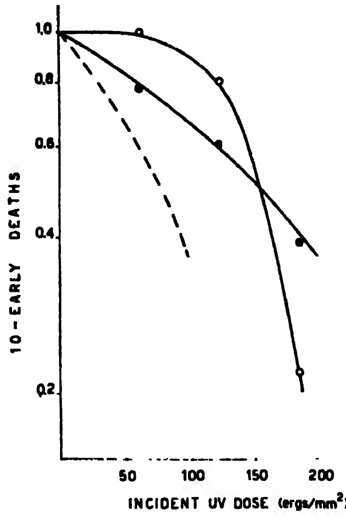

Fig. 2. Dose-effect relations for *Habrobracon* eggs dying before blastoderm formation after treatment with ultraviolet radiation. ● unfertilized; ○ fertilized; dashes, theoretical curve for a doubling of the target size of a haploid nucleus.

If mitotic inhibition can be induced by more than one mechanism and if, as appears probable, the nucleolus is not necessary for early development in *Drosophila*, then it is possible to resolve the paradox that grew out of (*1*) the nucleolar ultraviolet irradiation experiment of Gaulden and Perry[36] where mitosis was inhibited in grasshopper neuroblasts and (*2*) the genetic removal of the nucleolus in *Drosophila* eggs where mitosis was not inhibited (Von Borstel and Rekemeyer[37]). In accord with the concept of multiple origins for ultraviolet radiation inhibition of mitosis, Uretz *et al.*[38] have found that mitosis also is inhibited by ultraviolet irradiation of the centromeres. Though mitotic inhibition by ultraviolet light is shown by the nuclear reactivation experiment to have a quantitatively different response than X-radiation, it is not known to what extent ionizing radiation can affect nucleoli or centromeres, or how either ionizing or ultraviolet radiation affects the centrioles. These remain as problems for the future.

REFERENCES

1 K. C. Atwood, R. C. von Borstel and A. R. Whiting, *Genetics*, 41 (1956) 804.
2 D. R. Parker, *Univ. Texas Publ.*, 5914 (1959) 113.
3 R. C. von Borstel and M. L. Rekemeyer, *Genetics*, 44 (1959) 1053.
4 A. R. Whiting, S. Caspari, M. Koukides and P. Kao, *Radiation Research*, 8 (1958) 195.
5 R. C. von Borstel, *Nature*, 175 (1955) 342.
6 R. C. von Borstel, *Convegno di Genetica, Atti della V Riunione Scientifica della Associazione Genetica Italiana*, 5 (1960) 35.
7 J. Brachet, *Experientia*, 9 (1953) 182.

[8] T. T. PUCK AND P. I. MARCUS, *J. Exptl. Med.*, 103 (1956) 653.
[9] R. C. VON BORSTEL, *Genetics*, 45 (1960) 1015.
[10] B. P. SONNENBLICK, *Proc. Natl. Acad. Sci. U.S.*, 26 (1940) 373.
[11] J. G. CARLSON, *Radiation Biol.*, 1 (2) (1954) 763.
[12] W. ST. AMAND, *Radiation Research*, 5 (1956) 65.
[13] E. NOVITSKI AND D. L. LINDSLEY, *Drosoph. Inform. Serv.*, 24 (1950) 90.
[14] E. NOVITSKI, *Genetics*, 36 (1951) 267.
[15] O. A. TROWELL, *J. Pathol. Bacteriol.*, 64 (1952) 687.
[16] E. F. OAKBERG, *J. Exptl. Zool.*, 134 (1957) 343.
[17] S. P. HICKS, *J. Cellular Comp. Physiol.*, 43, suppl. 1 (1954) 151.
[18] E. F. OAKBERG, *Proc. X Intern. Congr. Genetics, Montreal*, 2 (1958) 207.
[19] A. R. WHITING, *J. Exptl. Zool.*, 83 (1940) 249.
[20] D. S. GROSCH AND R. L. SULLIVAN, *Radiation Research*, 1 (1954) 294.
[21] W. A. CREASY AND L. A. STOCKEN, *Biochem. J.*, 69 (1958) 17P.
[22] S. WOLFF AND H. E. LUIPPOLD in J. S. MITCHELL, B. E. HOLMES AND C. L. SMITH, (Editors), *Progress in Radiobiology*, 1956, Oliver and Boyd, Edinburgh, 1956, p. 217.
[23] S. WOLFF, *Nature*, 173 (1954) 501.
[24] F. H. SOBELS, *Intern. J. Radiation Biol.*, 2 (1960) 82.
[25] D. S. GROSCH, *Radiation Research*, 9 (1958) 123
[26] S. WOLFF AND K. C. ATWOOD, *Proc. Natl. Acad. Sci. U.S.*, 40 (1954) 187.
[27] L. E. LA CHANCE, *Radiation Research*, 11 (1959) 218.
[28] R. L. AMY AND R. C. VON BORSTEL, *Proc. 2nd Intern. Photobiol. Congress, Turin*, 1957, p. 419
[29] R. C. VON BORSTEL, in A. TYLER, R. C. VON BORSTEL AND C. B. METZ (Editors), *The Beginnings of Embryonic Development*, Am. Assoc. Advance Sci., Washington, 1957, p. 175.
[30] R. W. ROGERS AND R. C. VON BORSTEL, *Radiation Research*, 7 (1957) 484.
[31] A. W. KIMBALL, *Biometrics*, 9 (1953) 201.
[32] G. E. MAGNI, *Radiation Research*, Suppl. 1 (1959) 347.
[33] K. C. ATWOOD, *Proc. 1st Intern. Photobiol. Congress, Amsterdam*, 1954, p. 143.
[34] K. C. ATWOOD AND F. MUKAI, *Am. Naturalist*, 88 (1954) 295.
[35] R. C. VON BORSTEL AND S. WOLFF, *Proc. Natl. Acad. Sci. U.S.*, 41 (1955) 1004.
[36] M. E. GAULDEN AND R. P. PERRY, *Proc. Natl. Acad. Sci. U.S.*, 44 (1958) 553.
[37] R. C. VON BORSTEL AND M. L. REKEMEYER, *Nature*, 181 (1958) 1597.
[38] R. B. URETZ, W. BLOOM AND R. E. ZIRKLE, *Science*, 120 (1954) 197.

MITOTIC EFFECTS OF MONOCHROMATIC ULTRAVIOLET IRRADIATION OF THE NUCLEOLUS

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Intense irradiation of one of the two nucleoli of the grasshopper neuroblast with a microbeam of ultraviolet radiation was shown by Gaulden and Perry[1] to inhibit mitosis. Using a high-pressure mercury arc lamp (General Electric AH-6) as a source with filters to remove most of the intense infrared, visible, and near ultraviolet radiation, they found that a 3-sec exposure (*ca.* 0.03 erg μ^{-2}) of one nucleolus to a microbeam 3 μ in diameter permanently stopped mitosis when the cell was in any stage from late telophase through the first half of middle prophase. During the second half of middle prophase the cell became gradually less affected by irradiation of a nucleolus and by late prophase was completely insensitive to it. Microbeam irradiation of a non-nucleolur region of the nucleus caused some delay but the cell usually divided within 24 h.

Nucleoli in the preceding study received a mixture of different wavelengths of ultraviolet radiation. In the present study effects of specific wave lengths were examined in order to determine whether proteins or nucleic acids were responsible for the mitotic delay induced in different stages of mitosis.

Embryos of the grasshopper, *Chortophaga viridifasciata*, were oriented in hanging drops so that the neuroblasts situated on the ventral surface of the embryo were uppermost against a quartz cover. The culture medium used was the glycine–glutamic acid solution described by Shaw[2]. The nuclear diameter is large in relation to the size of the neuroblasts; therefore in a small proportion of the cells at least one of the two nucleoli will, purely by chance, lie close to the cover. This is important in order to insure that there is little protoplasmic material to absorb the ultraviolet radiation before it reaches the nucleolus. Nucleoli selected for irradiation were within 7.5 μ of the quartz cover in the earlier experiments, and within 5 μ in the later ones.

The microbeam apparatus used was the type designed by Uretz and Perry[3]. Monochromatic radiation was obtained by focussing the beam of a high pressure mercury arc lamp (General Electric AH-6 or Philips SP-500) on the entrance slit of a quartz monochromator. The image of the exit slit was focussed on the primary aperture of the microbeam apparatus. This aperture was brought to a focus as a microspot in the plane of focus of the viewing microscope of the microbeam apparatus and centered laterally on cross hairs of the viewing microscope. The aperture used in these experiments gave a microspot 3 μ in diameter. This is slightly smaller than the diameter of the

* Operated by Union Carbide Corporation for the U.S. Atomic Energy Commission.

References p. 253

nucleolus. Photoreactivation was avoided by passing all microscope illumination through appropriate filters. Ultraviolet intensity was measured at the beginning and end of each day's experiments by inserting under the reflecting objective of the microbeam apparatus an end window photomultiplier with a uranium glass cover that converts the ultraviolet to radiation of longer wavelengths detectable by the photomultiplier.

Results obtained for each mitotic stage and for wavelengths 2650 and 2804 Å are expressed as the time required for the controls to reach metaphase divided by the time required for the treated cells to reach metaphase. A ratio of 1.0 indicates, therefore, no effect; a ratio of zero means that the treated cell failed to divide at all during the period of observation (usually several days); and a ratio, for example, of 0.67 means that the treated cell took one and one-half times as long as the untreated one to reach metaphase. The efficiencies of the 2650 and 2804 Å wavelengths in retarding mitosis, that is, in increasing the interval of time required by the cell to reach metaphase, are expressed as the inverse of the dose required to increase this time interval to one and one-half times that of the unirradiated controls.

For all of the mitotic stages studied prior to early prophase, 2650 Å was more effective than 2804 Å in retarding mitosis. In early prophase, however, there was overlapping of the 70% confidence limits, and middle prophase showed no difference in the efficiencies of the two wavelengths. This suggests either a gradual loss of RNA sensitivity or RNA effectiveness in altering the mitotic rate as prophase progresses.

The neuroblast was most sensitive mitotically to 2650 Å and 2804 Å at middle telophase and very early prophase. Both of these stages are characterized by striking changes within the nucleus. During middle telophase the new nuclear membrane is formed, the nucleoli increase rapidly in size, the chromosomes gradually change in appearance from threads to granules, and DNA synthesis begins (Gaulden[4]). At very early prophase the granular appearance of the living nucleus is giving way to the combination of threads and granules that will later be replaced by an exclusively thread-like chromosomal material.

Early experiments, in which the time required by each irradiated cell to reach metaphase was compared with a median time determined from all the control cells, gave some indication that a small dose of ultraviolet radiation given to a nucleolus might stimulate rather than retard mitosis. In order to test this, two mitotic stages, early prophase and middle prophase, were selected. Since the time intervals from these stages to metaphase are short and slight differences in the exact stage of the treated and control cells could bias the results, individual controls were used. Two cells in the same embryo at exactly the same point of development within a given mitotic stage were located; then one was treated and both were timed to metaphase. Of 12 cells in early prophase given small doses of 2804 Å 10 reached metaphase ahead of their controls and 2 after their controls. Of 8 cells in middle prophase given small doses of 2804 Å the 7 with the smallest dose reached metaphase ahead of their controls and the remaining one reached it at the same time. On the other hand, 2650 Å showed no such accelerating effect on mitosis. In both early and middle prophase the ratios of control to treated cell division times were about equally scattered above and below 1.0. It appears from these results that the protein rather than the RNA is the effective component of the nucleolus in producing ultraviolet-induced acceleration of mitosis. Why this should be true we have no evidence at present.

When both nucleoli were irradiated, the mitotic retardation produced by a given dose to each nucleolus corresponded to what would be expected if only one nucleolus had been irradiated with that dose. In other words, the mitotic retardation is approximately the same, whether a given dose is administered to one or to each of the two nucleoli in a cell. This was true for all stages and for both wavelengths.

The effect of microbeam irradiation of the nucleus at some distance from either of the nucleoli has been tested for the different mitotic stages studied and for both wavelengths used. In general the larger doses retard or stop mitosis and the smaller ones have less effect, but the points, when plotted, show a much greater spread than for nucleolus irradiation. This suggests that the effect resulting from non-nucleolus irradiation may vary considerably with the particular non-nucleolar part of the nucleus absorbing the radiation. It might vary from one part to another of a given chromosome and from chromosomal to interchromosomal region.

It has long been known that the nucleolus consists mainly of protein and ribonucleic acid (Vincent[5]). In recent years it has come to be increasingly recognized that ribonucleic acid and ribonucleoprotein play essential roles in protein synthesis. Certain lines of evidence suggest that in the grasshopper neuroblast the metabolic activities of the nucleolus are necessary for completion of two-thirds of the mitotic cycle (including interphase). *(1)* The neuroblast must be supplied with extracellular growth substances for normal mitotic progress of stages from telophase to midprophase (Gaulden and Kokomoor[6]); since the nucleolus incorporates tritiated cytidine in these stages (Gaulden and Perry[1]), it may be directly involved in the utilization of these substances. *(2)* Mitosis can be stopped almost immediately by irradiation of one nucleolus. Experiments are in progress to investigate further at the different mitotic stages the relation of the nucleolus to synthesis of protein and nucleic acids.

REFERENCES

1 M. E. Gaulden and R. P. Perry, *Proc. Natl. Acad. Sci U.S.*, 44 (1958) 553.
2 E. I. Shaw, *Exptl. Cell Research*, 11 (1956) 580.
3 R. B. Uretz and R. P. Perry, *Rev. Sci. Instr.*, 28 (1957) 861.
4 M. E. Gaulden, in H. S. Ducoff and C. F. Ehret, (editors), *Mitogenesis*, Univ. of Chicago Press, 1956, pp. 38,39.
5 M. S. Vincent, *Intern. Rev. Cytol.*, 4 (1955) 269.
6 M. E. Gaulden and K. L. Kokomoor, *Proc. Soc. Exptl. Biol. Med*, 90 (1955) 309

MECHANISM OF PHOTODYNAMIC ACTION AS ILLUSTRATED BY *IN VITRO* SYSTEMS

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Since 1900, when Raab first discovered the photodynamic inactivation of bacteria, the effects of dye and light on many biological systems have been studied. Despite many varied observations, however, the mechanism underlying photodynamic action has not been fully elucidated, although it was known that the presence of oxygen is necessary and that a variety of fluorescent dyes can act as sensitizers for the process.

Since biological systems are extremely complex and do not readily lend themselves to a physico-chemical analysis of the mechanisms underlying photodynamic action, we elected to use an organic compound as a substrate for these investigations. *p*-Toluenediamine (PTD) dissolves in water to give a colorless solution. On oxidation a brown compound is formed. Autoxidation takes place readily at alkaline pH's, and only very slowly at pH 7, where our studies were undertaken. When to a solution of PTD at pH 7, one adds a dye such as proflavine, the rate of autoxidation of the PTD is in no way affected, if the solution is kept in the dark. Upon illumination with visible light, however, the solution turns brown almost immediately. Depriving the solution of oxygen completely abolishes the photodynamic effect. The rate of the photo-sensitized oxidation of the amine was followed quantitatively by determining the rate of increase of optical density at the absorption maximum of the brown oxidized compound. In this fashion the rate of photo-sensitized oxidation of PTD was measured as a function of dye concentration, substrate concentration, oxygen concentration, light intensity and pH. The data obtained fit the following scheme of reaction:

(*1*) $D + h\nu \rightarrow D^*$	Absorption of visible light to give dye molecules in the first singlet electronically excited state.
(*2*) $D^* \rightarrow D + h\nu_f$	Fluorescence and/or heat or internal conversion to ground state.
(*3*) $D^* \rightarrow D'$	Transition to long-lived state.
(*4*) $D' \rightarrow D + h\nu_p$	Phosphorescence and/or heat or internal conversion to ground state.
(*5*) $D' + D \rightarrow 2D$	Concentration quenching of long-lived state.
(*6*) $D' + O_2 \rightarrow DO_2$	Formation of "photoperoxide"
(*7*) $DO_2 \rightarrow D + O_2$	Spontaneous decomposition of "photoperoxide".
(*8*) $DO_2 + D \rightarrow 2D + O_2$	Concentration quenching of "photoperoxide".
(*9*) $DO_2 + S \rightarrow D + SO_2$	Oxidation of substrate

Assuming steady-state concentrations for the transient species D^*, D' and DO_2, we

obtain for the quantum yield of the photo-sensitized oxidation of substrate the expression.

$$\Phi = \frac{k_3}{k_2+k_3} \cdot \frac{k_6(O_2)}{k_4+k_5(D)+k_6(O_2)} \cdot \frac{k_9(S)}{k_7+k_8(D)+k_9(S)}$$

In dilute solutions of proflavine the quantum yield is 3.0. Comparing this expression with the experimental results, we find that $k_5/k_6 = 0.60$; thus a long-lived dye molecule has almost equal probability of being de-activated by an unexcited dye molecule and of reacting with oxygen. This suggests that every encounter of a molecule with D′ molecules leads to a reaction, and the lifetime of the D′ molecules can therefore be estimated from the observed ratio k_6/k_4, divided by the number of diffusional collisions in water at room temperature, as $3 \cdot 10^{-4}$ sec. In contrast, the lifetime of the first electronically excited state of the dye (D*, the fluorescent state) has been estimated as $5 \cdot 10^{-9}$ sec. Similarly we can estimate the lifetime of the dye-peroxide complex to be $1.4 \cdot 10^{-4}$ sec.

The most important points at which our scheme differs from those previously postulated are the following:

(*1*) Our rate data necessitate the postulation of a complex between excited dye molecules in the long-lived state and oxygen; for convenience we have termed this a "photoperoxide". Suggestions by previous workers involving the direct excitation of oxygen molecules by excited dye molecules can be objected against on various energetic grounds. Although direct proof of a complex such as we postulate is difficult to obtain, this would be an energetically feasible phenomenon.

(*2*) Participation in the reaction of dye molecules in the long-lived (presumably triplet) state. Thus, in contrast to previous suggestions concerning the participation of the fluorescent (first electronically excited singlet) state, we have shown that photoxidation involves the participation of molecules in the long-lived state. The property of fluorescence, therefore, is not sufficient to guarantee the effectiveness of a dye as a photosensitizer. And indeed, we have found this to be the case: only those dyes which can form long-lived dye molecules are able to act as sensitizers in photodynamic action. In conformity with previous investigations we have termed such compounds photoreducible dyes: the reduction of these dyes by means of light and a mild reducing agent such as ascorbic acid, allylthiourea or EDTA occurs via the participation of dye molecules in the metastable long-lived excited state. We have found that only those dyes which are photoreducible (and which, therefore, are able to form dye molecules in the long-lived excited state) can act as sensitizers in photoxidation. Such dyes belong to the following families of dyes: Thiazines (*e.g.*, methylene blue), Xanthenes (*e.g.* fluorescein), Thiazoles (*e.g.* thioflavin TG), Acridines (*e.g.* proflavine), Azines (*e.g.* neutral red), Porphyrins (*e.g.* hematoporphyrin), and Riboflavin. Among dyes which are inactive are Azo dyes (*e.g.* Congo red), Oxazines (*e.g.* nile blue), Indamines (*e.g.* tolulene blue), Anthraquinones (*e.g.* alizarin red), Cyanines (*e.g.* pinacyanol), Nitro dyes (*e.g.* Martius yellow) and Indophenol. Triphenylmethane dyes, such as crystal violet are photoreducible only when they are bound to a high-polymeric substrate, such as DNA, and can act as sensitizers for photoxidation only when in the bound state.

Following the elucidation of this mechanism for photodynamic action we desired to find out whether the same mechanism would hold for a biological substrate. To this end we investigated the photosensitized inactivation of a transforming principle, since it is relatively more simple than most other biological substrates. DNA inducing resistance to streptomycin in *D. pneumoniae* was prepared and assayed by the usual methods. For the study of its photodynamic inactivation, $5 \cdot 10^{-6}$ *M* dye was added to the DNA in saline. Two solutions (with and without dye) were kept in the dark, and two identical ones were illuminated at a distance of 15 cm from the front surface of a 500-W projector. No rise in temperature occurred in any of the illuminated samples. All irradiations were performed in the presence of atmospheric oxygen. Following illumination the transforming activity of the DNA was assayed at three different concentrations, all at a level where previous assays had shown the transforming activity to be independent of DNA concentration. The concentration of dye in contact with the cells was $5–10 \cdot 10^{-7}$ *M* and total cell counts showed that at this concentration none of the dyes impaired cell viability. In no case did illumination in the absence of dye result in a change in transforming activity. In those cases where inactivation did occur, the remaining activity was in all cases still proportional to DNA concentration at the three concentrations used for assay.

Fig. 1 shows that after fifteen minutes' irradiation in the presence of methylene blue the DNA has lost half of its transforming activity. In the absence of light no

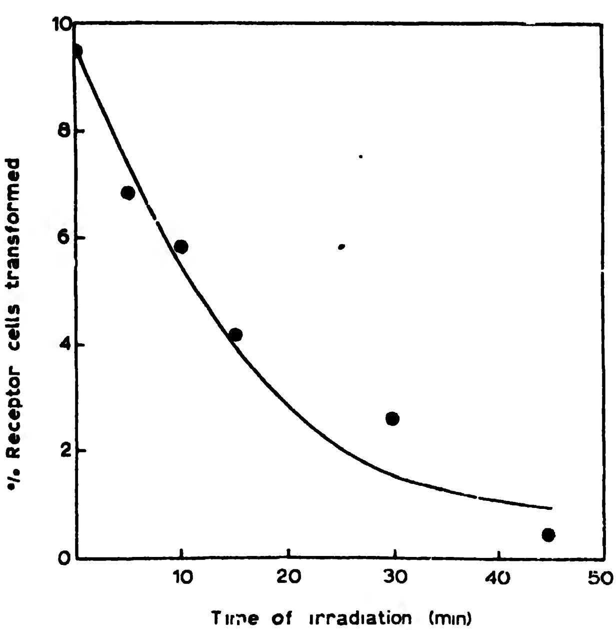

Fig. 1.

inactivation occurred. Table I lists the results for fifteen minutes' irradiation using various dyes as sensitizers. Rose bengal, methylene blue, proflavine, neutral red and riboflavine are effective sensitizers for the photodynamic inactivation, as is crystal violet, since it is bound to the DNA. Cresyl violet, methyl orange, and alizarin red are ineffective. Both proflavine and thioflavine TG reduce the transforming activity

(without bactericidal activity at the low concentrations employed) even in the absence of light, thus preventing us from noting their expected photosensitizing action. This "dark effect" may be related to the known mutagenic activity of acriflavine, of which proflavine is the principal constituent. In all cases, the ability or inability of

TABLE I

PHOTODYNAMIC INACTIVATION OF TRANSFORMING PRINCIPLE

Dye	*Transforming activity (% of activity of control containing no dye)*	
	Dye alone	*Dye and light*
Rose Bengal	100.5	59.8
Methylene Blue	95.6	44.0
Proflavine	68.2	64.2
Neutral Red	110.1	40.6
Crystal Violet	101.4	72.1
Thioflavine TG	71.0	63.2
Cresyl Violet	105.3	93.0
Methyl Orange	100.5	98.9
Alizarin Red	96.2	97.9
Riboflavine	89.9	51.9

the dyes to sensitize the photodynamic inactivation of transforming principle is paralleled by their ability to sensitize the photoxidation of PTD and by their photoreducibility. Here again, therefore, only those dyes which can form molecules in the long lived excited state can act as sensitizers, and one may presume, therefore, that the mechanism elucidated for the photoxidation of PTD may hold true for the photosensitized inactivation of the transforming principle as well.

As a third step in our investigation we are studying the photodynamic inactivation of tumor cells *in vitro* and *in vivo*. The tumors tested included the Gardner Lymphosarcoma, (6C3HED), Sarcoma 180, Ehrlich ascites and a mammary carcinoma, (dbrB), all grown in mice. Cell suspensions were prepared in saline and adjusted to pH 7.4, from rapidly growing solid tumors. These suspensions were diluted to give 10^6 cells per ml, dye was added, usually in 10^{-5} *M* concentration, and the suspensions were illuminated at room temperature, in the same manner as in the case of the transforming principle. After illumination the suspensions were centrifuged and the cells washed with saline and resuspended in saline containing 200 units of Penicillin. Sterile precautions were observed throughout. Each suspension was tested by the injection of 0.5 ml, subcutaneously in four different sites in four different mice.

Fig. 2 illustrates data obtained in the case of neutral red and Gardner Tumor cells. The ordinate represents the number of days the mice were free of tumor (*i.e.*, the number of days until a tumor could be palpated; death usually occurred about 10 days later). Along the abscissa are plotted in succession two controls containing no dye, and two samples containing neutral red, in each case one kept in the dark and one illuminated. It is evident that in the absence of dye illumination had no effect on the viability of the tumor cells. Similarly, the presence of dye *per se* had no effect. Illuminating the cell suspension in the presence of dye for 30 min, however, completely

inactivated the tumor cells, since they were unable to elicit tumor formation even after thirty days.

As shown in Table II, results similar to these were obtained using proflavine, acridine orange, eosin, thionine, and methylene blue. Rose bengal, crystal violet, janus green B, and 2,6-dichloroindophenol inactivated the tumor cells even in the absence of

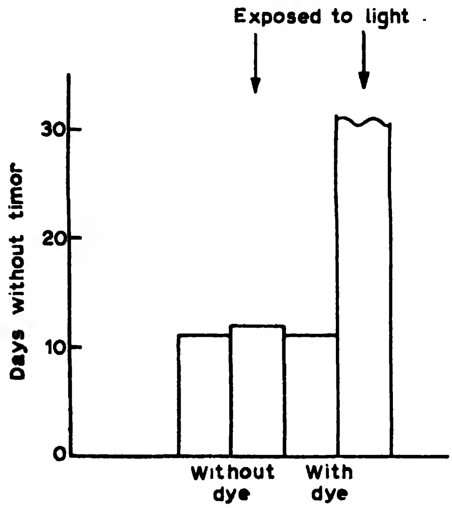

Fig. 2.

TABLE II

PHOTODYNAMIC INACTIVATION OF TUMOR CELLS *in vitro* (GARDNER LYMPHOSARCOMA)

Dye	Viability (Number of days until tumor palpable)			
	No dye		With dye	
	No light	Light	No light	Light
Rose Bengal	11	11	30+	30+
Eosin	11	12	15	30+
Methylene Blue	10	11	11	30+
Thionine	11	12	13	30+
Proflavine	9	10	10	30+
Acridine Orange	11	12	12	30+
Neutral Red	11	12	14	30+
Crystal Violet	11	12	30+	30+
Thioflavine TG	11	12	30+	30+
Janus Green B	11	11	30+	30+
Indophenol	11	12	30+	30+
Methyl Green	11	12	18	30+
Congo Red	11	11	12	12

light. Congo red had no effect, either in the dark or upon illumination. Results similar to the foregoing were obtained using Sarcoma 180, Ehrlich ascites and a mammary carcinoma (dbrB). As in the case of the photosensitized autoxidation of PTD and of the photodynamic inactivation of transforming principle, therefore, only those dyes which are capable of being photoreduced are able to act as sensitizers for the photodynamic inactivation of tumor cells.

RENDEMENT QUANTIQUE DE LA RADIATION 2537 Å SUR UN ADN BACTÉRIEN ET DES BACTÉRIOPHAGES

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Nous avons étudié le rendement quantique de la radiation 2537 Å sur des acides désoxyribonucléiques bactériens et viraux. Nous avons d'abord calculé l'absorption d'une molécule d'ADN. Le coefficient d'absorption de nos solutions était de $2.13 \cdot 10^4$. Pour une portion de molécule d'ADN de poids moléculaire 10^6 la surface projetée correspondante est de 570 mμ^2. Cette même portion correspond à environ 2850 nucléotides, chacun ayant comme surface projetée perpendiculairement à l'axe de la fibre 0.2 mμ^2.

Dans le cas de solutions très diluées, on peut considérer que toutes les molécules d'ADN sont distinctes. Elles tendent à offrir au rayonnement leur surface projetée. Pour une épaisseur de 1 cm, la concentration correspondant à une couche uniforme de 1 molécule est de:

$$\frac{1 \text{ cm}^2}{0.2 \text{ m}\mu^2} = \frac{10^{14}}{0.2} = 5 \cdot 10^{14} \text{ nucléotides/cm}^3$$

$$\text{ou } 2.9 \cdot 10^{-7} \text{ g/cm}^3 \text{ d'ADN}$$

Une telle solution sous 1 cm d'épaisseur doit avoir la même absorption qu'une monocouche, c'est à dire qu'une molécule d'ADN. Par extrapolation de la courbe de densité optique en fonction de la concentration on a, pour cette solution, une densité optique 0.0061.

Donc, pour la radiation 2537 Å, une molécule ou fraction de molécule d'ADN *absorbe 13 p. 1000 des photons qu'elle reçoit.*

Nous avons irradié dans des conditions semblables un ADN bactérien et des bactériophages. L'ADN bactérien est un ADN transformant du Pneumocoque dont nous avons suivi l'aptitude à transmettre le caractère résistance à la Streptomycine à une souche sensible. Les bactériophages étudiés sont le ΦX174 et le S. 13 dont l'ADN a une structure en simple hélice, et aussi le T_2 dont l'ADN est constitué d'une double hélice. Les irradiations U.V. ont été effectuées en solution de NaCl 0.15 *M*. Les irradiations X ont été faites en milieu protecteur contre les effets indirects: les échantillons ont été dilués dans de l'extrait de levure à 10%, puis congelés à —50° et irradiés. Les différents résultats obtenus sont donnés dans le Tableau I.

* Avec la collaboration technique de Mademoiselle M.-L. DESVOYE.

TABLEAU I

		T_2	$\Phi X174$, S.13	TP
Poids d'ADN (g)		$2.3 \cdot 10^{-15}$	$4 \cdot 10^{-18}$	$1.2 \cdot 10^{-19}$
U.V. $D_{0.37}$ (ergs·mm^{-2})		40	200	80,000
Photons absorbés pour 1 choc efficace	par ADN	5250	460	5,200
	par nucléotide	0.013	0.07	26
Rayons X $D_{0.37}(10^3R)$	à 2°	40	250	1,200
	à 50°		420	4,000
Ionisations par ADN pour 1 choc efficace	à 2°	20	2	
	à −50°		3.5	1
Photons absorbés / ionisations		260	130	5,200
U.V. Joules absorbés par mg/ADN pour $D_{0.37}$		0.017	0.09	34

Ces résultats préliminaires montrent que les ADN simple et double des bactériophages se comportent à peu près de la même façon en donnant des rendements quantiques du même ordre. L'ADN transformant est beaucoup plus résistant aux U.V. et a un rendement quantique 30 fois plus faible. Il faut attendre d'autres expériences pour expliquer ces différences.

MODIFICATION OF RADIATION DAMAGE AND ELECTRON SPIN RESONANCE MEASUREMENTS IN *VICIA FABA* SEEDS*

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I am happy to be able to deliver this short report on our current investigations on *Vicia faba* seeds. Our interest is centered around X-ray damage in the seeds, especially when very dry. We are trying to draw some indirect information on earlier steps involved in the radiation effect, by studying the modification in damage resulting from a series of different treatments applied after irradiation. In addition we are also trying to get more direct information about these earlier steps by means of a very recent physical technique, that is, electron-spin resonance absorption measurements in the irradiated material. Following up these lines, we have also studied the influence of illumination by fluorescent light, this being where photobiology itself comes into the report.

MATERIAL AND BASIC FACTS

Vicia faba, as is well known, is a very large bean needing much space in irradiation and growth experiments. In order to be able to irradiate a reasonable number of seeds at the same time we are using a special variety, *Vicia faba minor*, which has smaller seeds and therefore suits our purposes well. The advantage of using the smaller seeds can be seen clearly from the first slide***.

Several authors, *e.g.* Ehrenberg[1] at Stockholm and Caldecott[2] at Brookhaven, reported some years ago that the radiation sensitivity of very dry seeds is much higher than that of seeds with medium water content. These data were given only for barley. We have however been able to confirm them in our own *Vicia faba minor* seeds of different progenies and under different experimental conditions[3]. The next slides*** illustrate this for beans of 2 moisture contents, namely 8.6% and 11.7%, irradiated with 3,000 R, and grown in saw-dust in the light. It is obvious that the dry beans are damaged severely by the irradiation, while in the seeds of higher moisture content radiation damage is comparatively small.

ESR-MEASUREMENTS

Searching for the cause of this sensitizing effect of drying, Zimmer and Ehrenberg started electron spin resonance measurements in irradiated embryos and whole seeds of different water content[4-6]. They were soon followed by our own group[7,8]. By means of this technique, the details of which time does not permit, we were able to show that

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*** Given at the Congress only.

References p. 265

in very dry embryos of *Vicia faba minor* seeds, 10,000 R of X-rays induces a detectable amount of free radicals, about $7 \cdot 10^{15}$ per gram dry weight, while in embryos of medium water content, no such radicals could be found (sensitivity about 10^5 radicals per gram dry weight). Investigating cotyledons and testae separately, we found that similar concentrations of free radicals were induced by the irradiation in both the cotyledons and the embryo. In testae, however, many more free radicals could be detected.

Following up this phenomenon we found evidence that even simple illumination induces free radicals in the testa of *Vicia faba minor*, not only in dry material but in some of medium water content as well. The typical esr-absorption curves shown demonstrate this fact (Fig. 1). The measurements were carried out in the physics

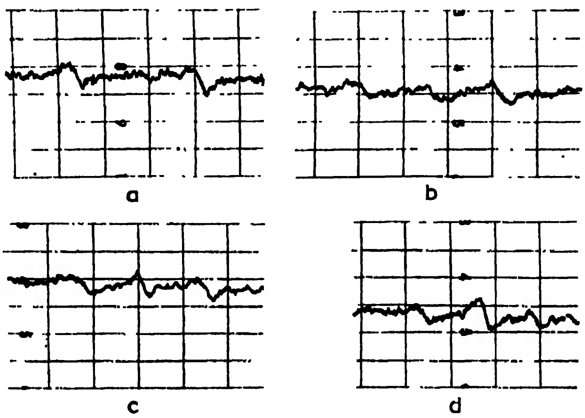

Fig. 1. Esr-absorption in testae after different illumination periods. Water content of testae 11.0%. Illumination with 4500 lux for (a) 0 h, (b) 13 h, (c) 1 day and (d) 2 days. 2 marker absorption peaks, one on each side of the free radical absorption.

department of Giessen University by my colleague Diplom-Physiker Von Foerster, who has designed a very sensitive esr-spectrometer and has spent a great deal of time carefully calibrating and adjusting it for these purposes. He employed a special marking substance displaying two peaks of absorption, one on each side of the free radical absorption to be expected. This was introduced into the cavity together with the biological sample. Thus standard comparisons and calculations of free radical contents were possible. The water content of the shells was 11.0%. They were separated from the beans and exposed to fluorescent light of about 4500 lux for 0 h, 13 h, 1 day, and 2 days respectively. It can be seen that the concentration of illumination-induced free radicals rose from uncertain amounts, in the beginning, to about twice the amplitude of the marker absorption peaks.

We feel that these findings are important in several respects. They show that, for esr-measurements on X-rayed biological material great care must be taken to exclude disturbing influences from uncontrolled factors such as daylight. They might also prove of relevance for all those investigations on light-dark reversal phenomena in seed germination on which an overwhelming amount of literature has been compiled during the last 10 years (see refs.[9,10] for literature). Specialists will perhaps remind us

that bean seeds are usually considered as light indifferent in their germination behaviour. However, recent investigations of Lane and Butler[11,12] revealed some photoformative effects in *Vicia* seeds in which short light exposures of the seeds caused alterations in the later development of the shoot, but, I would rather not stress such ideas as long as simpler explanations for the effects observed are still at hand. One such simpler explanation might be that melanins, as in a number of zoological subjects studied by Mason and co-workers recently[13,14], are the cause of the extreme esr-absorption tendency in our material, being transformed by illumination or X-irradiation into free radical states.

POSTTREATMENT

A recent paper by Curtis and co-workers[15] at Brookhaven must be mentioned, in connection with posttreatment of X-irradiated seeds in order to get indirect information on initial steps involved in radiation damage. The authors, working with normal and very dry barley seeds, reported that very dry seeds show the same damage

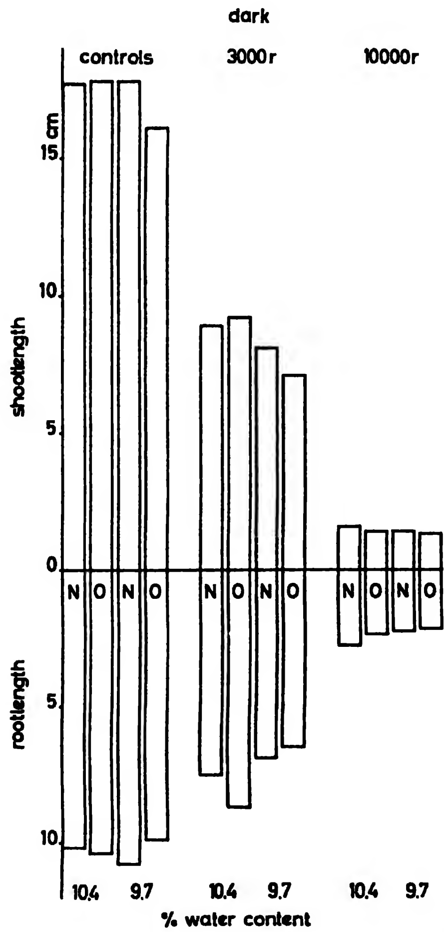

Fig. 2. Shoot and root length of seedlings 11 days after soaking, grown in the dark. Seeds of two moisture contents, 9.7% and 10.4 %. Doses of 3,000 R and 10,000 R X-rays. Soaking 4 min after end of irradiation, under aerobic (O) or anaerobic (N) conditions. Mean from 60 beans in each case.

References p. 265

as seeds of medium water content if germinated immediately after irradiation. Thus, there is no sensitization by the drying process. According to the authors it is only the time factor, that is prolonged storage after irradiation, which brings about the higher damage in the dry seeds. In their experiments, soaking was done under anaerobic conditions for an initial 1-h period and the seedlings were grown in the light.

We have tried to check these findings in our *Vicia* seeds to get more insight into the factors involved in this effect, but were unable to find anything corresponding to the data of Curtis and co-workers. In Fig. 2 the results of our experiments are summarized. Root and shoot lengths of the seedlings after 11 days of growth under strictly defined experimental conditions are indicated on the graph. Seeds of two moisture contents on irradiation are shown, namely 9.7% and 10.4%. They were given 3,000 R or 10,000 R of X-rays, apart from the controls, and were soaked 4 min after the end of irradiation for an initial period of 8 h under either aerobic or anaerobic conditions. They were then grown in sawdust. The results given are the mean values from 60 beans.

In contradiction to the other authors, radiation damage was very great indeed in these samples soaked 4 min after irradiation, while beans of higher water content, *viz. ca.* 12%, showed no damage at all. Statistics showed that apart from the irradiation and from moisture content itself no other treatment gave significant differences. There was no difference between aerobic and anaerobic soaking, nor had aerobic soaking any effect on the ultimate radiation damage, nor was any higher interaction significant. This is brought out more clearly by the following slide (Fig. 3, left side), where the X-ray data are presented in percent of individual controls.

When thinking of some possible explanation for the observed discrepancies between

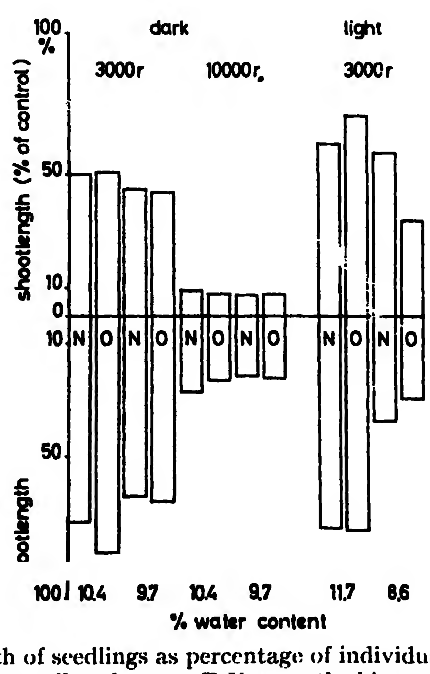

Fig. 3. Shoot and root length of seedlings as percentage of individual controls. Seeds of different moisture contents. Doses of 3,000 R and 10,000 R X-rays. Soaking 4 min after end of irradiation, under aerobic (O) or anaerobic (N) conditions. Growth in the dark (11 days) or in the light (12 days). Mean from 60 beans each.

our results and those of Curtis *et al.*, it occurred to us that Curtis and co-workers had grown their seedlings in the light while we, for practical reasons, had grown them in the dark. To check whether or not illumination had any influence on the results we have now run a corresponding experiment, growing our beans (after irradiation and soaking) in fluorescent tube illumination of about 4500 lux from the 3rd day onward by which time the seedlings had grown through the sawdust. Final measurements were taken on the 12th day of growth.

The results as percentage of individual controls are added here to the earlier data on the same graph (Fig. 3, right side). There were beans of 11.7% and 8.6% water content, given 3,000 R of X-rays and soaked either anaerobically or aerobically as before.

It can be seen from the graph that, with illumination an oxygen effect in the soaking procedure becomes obvious in the very dry beans, the damage being greater in aerobically soaked seeds than in anaerobically soaked seeds. There is still considerably greater damage in the dry seeds as opposed to the medium water content seeds if only roots are considered. For shoots grown in the light however the results of Curtis *et al.* would seem to be confirmed. That is, X-ray damage is not significantly greater in shoots of dry seeds than in shoots from seeds of medium water content provided that the soaking is done under anaerobic conditions.

I need not enlarge here on all the many interesting conclusions and speculations which might easily be derived from our results. I think speculations should be delayed until we have confirmed these findings further. We hope to do this very soon and we are all looking forward very much to what will emerge.

REFERENCES

1 L. Ehrenberg, *Botan. Notiser*, 108 (1955) 184; *Hereditas*, 41 (1955) 123.
2 R. S. Caldecott, *Radiation Research*, 2 (1955) 339; 3 (1955) 316.
3 W. Klingmüller, *Z. Naturforsch.*, 14b (1959) 268.
4 K. G. Zimmer, L. Ehrenberg and A. Ehrenberg, *Strahlentherapie*, 103 (1957) 3.
5 A. Ehrenberg and L. Ehrenberg, *Arkiv Fysik*, 14 (1958) 133.
6 B. Sparrman, L. Ehrenberg and A. Ehrenberg, *Acta Chem. Scand.*, 13 (1959) 199.
7 W. Klingmüller and M. C. Saxena, *Naturwissenschaften*, 46 (1959) 475.
8 W. Klingmüller, G. R. Lane, M. C. Saxena and D. J. E. Ingram, *Nature*, 184 (1959) 464.
9 M. Evenari, *Radiation Biol.*, 3 (1956) 519.
10 E. H. Toole, S. B. Hendricks, H. A. Borthwick and V. K. Toole, *Ann. Rev. Plant Physiol.*, 7 (1956) 299.
11 G. R. Lane and R. D. Butler, *Nature*, 181 (1958) 1080; 183 (1959) 1063.
12 R. D. Butler and G. R. Lane, *J. Linnean Soc. London, Botany*, 61 (1959) 170.
13 H. S. Mason, D. J. E. Ingram and B. Allen, *Arch. Biochem. Biophys.*, 86 (1960) 225.
14 M. Edwards, G. A. Kerkut, K. A. Munday, D. J. E. Ingram, M. C. Saxena and K. Leach, *Nature*, 184 (1959) 1402.
15 H. J. Curtis, N. Delihas, R. S. Caldecott and C. F. Konzak, *Radiation Research*, 8 (1958) 526.

ELECTRON DIFFUSION PROCESSES IN PHOTOBIOLOGY*

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The idea of diffusion or migration of electrons is of interest in several connections. One is when the absorption of an energetic photon leads to ionization of a molecule in a tissue and the appearance of a free electron; one wishes to know what can happen to this electron. There is evidence that in photosynthesis an electronic diffusion process takes place in the initial prechemical stage. Also a great many reactions in tissues involve transfers of electrons. It is customary to assume that these transfers take place directly only over a very short range, and that long-range movement is associated either with the movement of a distinct chemical species, or with the transfer of an electron through a series of distinct chemical species. It has been suggested at times, first by Szent-Györgyi[1] that it is not necessary to postulate a new chemical species for each step of the transfer, but that one might suppose instead that the electron migrates through the intervening space as a free particle.

This idea has not had general acceptance and is not even admitted as a reasonable hypothesis by many workers. Its failure must be ascribed in part to the fact that while a good theoretical model, the so-called band model, exists to account for the movement of electrons in metals and semiconductors, this model cannot be extended to the kind of system which is found in living tissues without severe and perhaps insurmountable difficulties. This statement must not be taken to mean that the diffusion of electrons cannot take place in such systems. Such diffusion must in general be possible. Any substance in which free electrons can be formed or into which they may be introduced, will exhibit electronic diffusion processes independently of whether the band model is applicable or not.

A brief description of the band model will be given here, with particular reference to organic substances. Some indication will be given of the limits of its applicability, and of what sort of picture can be used to think of electronic diffusion when the band model fails.

Let us consider, from a drastically simplified point of view, an organic molecule of moderate size. We shall suppose that this molecule has a well-defined number of states which can be occupied by electrons, and that each electron in the molecule must occupy such a state. In order to confine our discussion to essentials, we shall characterize the states by their energy, and set their total number to be equal to the number of electrons, n, plus one. Normally the n states of lowest energy will be occupied and the remaining state, lying a few electron volts above the highest occupied state, will

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be empty, as in Fig. 1A. It is possible to raise an electron from an occupied state into the uppermost one by the absorption of a photon of the correct energy, and a process of this sort is what we mean when we say that a molecule absorbs light.

Let us now suppose that instead of exciting an electron from a lower state into a higher one, we add an extra electron to the molecule, perhaps by letting it bind a free electron from an electron beam. Such a process is possible in general, and the binding energy will be of the order of a few electron volts. We must put this electron into the highest-lying state since it is the only one remaining, and its energy will be approximately the characteristic energy of this state.

We must now try to see the situation of this extra electron from its own point of view. It will occupy an orbit, so to speak, which is in rough approximation confined to the region of space occupied by the molecule. If we try to separate it from the molecule, we must give it more energy, and its energy must increase rather sharply as we move it away from the molecule. In other words, the electron sees the molecule as a potential well - -a hole into which it has fallen, and from which it cannot escape without help (Fig. 1B).

Let us now consider a large number of identical molecules arranged so that they are closely packed, regularly spaced, and similarly oriented; a crystal, in brief. An extra electron in one of these molecules now sees a large number of identical states which it might occupy if it were free to move. It would seem at first glance that it would be prevented from moving through the crystal by the fact that in passing from one molecule to the next, it would be necessary to separate it from the first molecule before putting it into the second, and that thus a large activation energy would be necessary to cause it to make a one-molecule step. Because of the so-called quantum mechanical tunnel effect, this is not in fact so.

The tunnel effect can be summarized briefly as one which allows a particle to pass through a barrier which it would find impossible to surmount because of a large energy requirement. A probability that the particle will be able to penetrate the barrier is associated with the effect, and this probability is related to the height and thickness of the barrier, and is very sensitive to them. For electrons it is of appreciable magnitude for distances of the order of a few angstroms and for barrier heights of the order of a few electron volts, which is the range of distances and energies with which we are concerned.

Because of the tunnel effect, the space available to the extra electron is not confined to the region occupied by the molecule, and if a molecule having an identical upper state is close enough, the electron may move into it without undergoing a jump in energy. In situations in which the probability of making such a transition is large, we say that the band model is applicable. In terms of this model, it is no longer correct to speak of N molecules each having a vacant upper state; we must think instead of the whole crystal having N vacant upper states. These states will not be identical in energy, but will occupy a more or less narrow band of energies; this is the so-called conduction band (Fig. 1C).

While the extra electron behaves as a free electron in having the whole crystal as its permitted range, it differs from a free electron in space in being more difficult to accelerate in an applied electric field. This sluggishness is associated with the degree of difficulty with which an electron can tunnel through the intermolecular barriers. We may retain the approximation that the electron is free by ascribing an "effective

References p. 270

mass" to it; the more difficulty the electron finds in passing through the barriers, the greater the effective mass. Similarly, as the barrier becomes less penetrable to electrons, the more nearly are we able to think of the molecules as individual molecules, separate from and unaffected by their neighbors. As the molecules become more nearly individuals, the character of the highest-lying state becomes more like that in an individual molecule. The total range of energies of upper states in the crystal becomes narrower, and thus the conduction band becomes narrower. We must therefore not think of the band property as being an absolute property of any crystal, but rather as a model which may be more or less applicable.

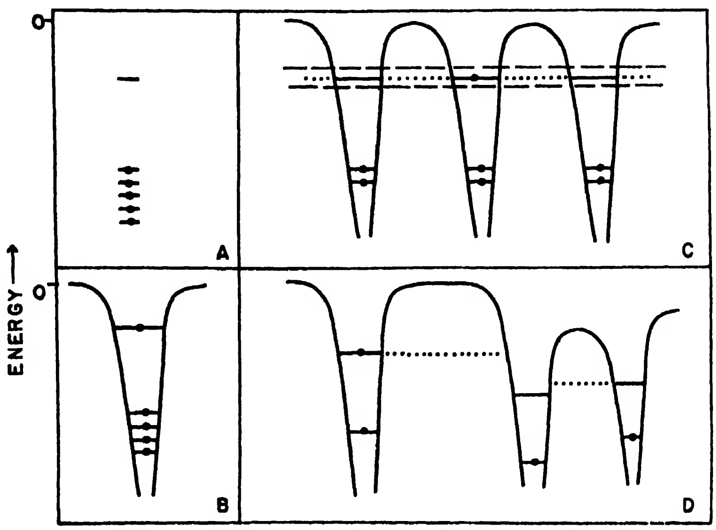

Fig. 1. Schematic representations of electron states in molecules and solids. See text for details.

The property of the band model which makes it useful as a mechanism for allowing energy to diffuse through a solid is simply the fact that there is a range of energy for which no allowed states of electrons exist. If energy is conveyed to an electron by exciting it to the upper state, the electron must retain that energy as long as it remains in the free mobile condition. It can carry the energy a considerable distance in the crystal and discharge it at some suitable site. Since such an electron is equivalent to a chemical reductant, we have introduced the possibility of separating oxidations and reductions spatially, as in the well-known Gurney-Mott[2] mechanism of the primary photographic process.

There are in particular two types of situations in which the band model is inappropriate, and these are of the sort which are of interest in biology. One is the case in which the probability of the electron being able to tunnel through the intermolecular barrier is small, causing the conduction band to become very narrow. Specifically, when the width of the conduction band approaches the value of the product of the Boltzmann constant and the absolute temperature, about 0.025 electron volts at room temperature, the model fails. This is because the requirement of identicalness of the molecules has become very stringent and hard to satisfy, and the differences in thermal energies of the molecules are sufficient to make the system effectively heterogeneous. This does not mean that it is no longer possible for an electron to tunnel from

one molecule to the next. It means rather that it is no longer correct to think of collective processes involving the whole array of molecules, and that we must now think of processes involving pairs of molecules and associate the electron with some particular molecule at a given instant. The tunnel effect may be still quite appreciable, and the electron may still diffuse through the array of molecules. The property of retaining its initial excitation energy until it loses it catastrophically still remains, and hence the potentiality of being able to account for diffusion of energy.

From a fundamental point of view this sort of "jump process" diffusion is quite distinct from the band model, but the differences do not change the essential character of the process from the standpoint of transfer of energy. The "jump" model seems more likely to be of use in biological systems since it is valid under circumstances lacking the high degree of regularity of structure which the band model requires. The writer[3] has offered evidence that such processes take place in the photoconductivity of certain dyes and pigments, and they may prove applicable in considering the initial process in photosynthesis.

A typical situation in biological systems is that of an array of non-identical molecules (Fig. 1D). Here an extra electron in a molecule will not in general be able to move to an adjacent molecule without losing or gaining energy. The band model is hardly applicable to systems of this sort. In principle, diffusion of an electron will still be possible since the tunnel effect still operates. A conceivable situation is that in which the electron passes through a series of molecules having successively lower allowed states. This situation offers no difficulties, and constitutes a channel for directed movement of energy. It may also be possible for an electron to move uphill in energy by acquiring thermal energy from the molecule in which it is localized, to enable it to occupy a higher-lying state of an adjacent molecule. If the molecules adjacent to each other are not greatly different chemically, the amount of energy required is likely to be relatively small.

We have based our discussion on the movement of an extra electron in a system of molecules. It is evident that such an electron can be produced by energetic ionizing photons. There is also considerable evidence that free electrons can be produced with photons of a few electron volts energy even in disordered systems by transfer from pigment molecules. The writer has found[4] that it is possible to sensitize photoconductivity in a borosilicate glass with organic dyes; that is, that an extra, mobile electron can be caused to appear in the glass with the expenditure of as little as 1.4 electron volts. Albrecht and Green[5] have performed experiments in which hydrocarbon glasses at 78°K became photoconductive by photoionization of tetramethylphenylenediamine using photons of 3.5 electron volts. Dye-sensitized photoconduction in proteins has been observed[1]. It does not seem to be out of the question that an electron could be set free to diffuse in a protein molecule by near-ultraviolet light absorbed by a tyrosine or tryptophane group. While the energy is much less than the ionization energy of tyrosine, only enough is required to move an electron into another bound state in which it is an excess electron. It will then be free to diffuse and to carry its excitation energy with it.

Recently some work done by Gordy and Patten[6] has given what is probably the best and most direct evidence for diffusion of electrons in proteins. They examined the electron spin resonance signals of X-irradiated proteins. When the irradiation took place at room temperature, it was found that all the unpaired spins were in

similar situations, probably at a cystine S-S bonds. When the irradiation and the examination were carried out at 78°K, the spins were found to be in a variety of situations; if the sample was now warmed to room temperature, all the spins were once more to be found at cystine sites. There seems to be clearcut evidence of an electronic diffusion process (probably in this case of an electron vacancy, or hole) which is frozen out at low temperatures, but is effective at room temperature.

In conclusion, it should be pointed out once more that the band model is not a necessary part of the hypothesis of electronic diffusion processes in biological systems; that such processes unquestionably exist in situations in which the band model is of doubtful validity, and that they deserve consideration in cases in which energy is supposed to migrate in a biological system.

REFERENCES

1. A. Szent-Györgyi, *Nature*, 157 (1946) 875.
2. R. W. Gurney and N. F. Mott, *Proc. Roy. Soc. (London)*, A164 (1938) 151.
3. R. C. Nelson, *Proc. of the Duke Conference on Electronic Conduction in Organic Solids*, (to be published).
4. R. C. Nelson, *J. Opt. Soc. Am.*, 50 (1960) 1029.
5. A. C. Albrecht and M. E. Green, *J. Chem. Phys.*, 31 (1959) 261.
6. Walter Gordy and F. Patten, (private communication).

Symposium 6

PHOTOREACTIVATION

Chairman: ARTHUR G. GIESE, Stanford, Calif. (U.S.A.)

Secretary: KJELD CHRISTENSEN, Copenhagen (Denmark)

INTRODUCTORY REMARKS

A. C. GIESE

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In his tribute to Niels Finsen at the opening session of this Congress, Dr. Hollaender indicated that considerable progress had been made at the Finsens Medicinske Lysinstitut in Copenhagen on the fundamental aspects of the action of ultraviolet radiations on cells. In the initial period following the discovery in 1877 of the bactericidal effect of sunlight by Downes and Blunt[1] in England it was not certain whether the effect of sunlight was upon the organism or upon the medium, whether oxygen was necessary for the effect, as in the case of photodynamic action, nor what wavelengths of sunlight were most effective. Bie in Finsen's laboratory showed that the radiation acted directly upon the organism since the medium became toxic only after a tremendous dosage of radiation whereas the organisms were killed by relatively small ones[2]. In another study he showed that oxygen was not necessary for U.V. action[3]. He also reported that the short band of ultraviolet radiations (U.V.) in arc light from 2000–2950 Å was 10–12 times as bactericidal as the much more intense long wavelengths of U.V.[4], a conclusion which Bang[5], at the same laboratory, supported with careful measurements of dosages. Hertel[6,7] in Germany, working with only a few wavelengths of the ultraviolet reported that the shorter the wavelength of the U.V. which he used, the greater its effectiveness, judged on the basis of dosages. At the same time many other interesting facts were discovered and techniques steadily improved. However, after the turn of the century interest in such studies waned.

It was the work of Gates at Harvard in the nineteen twenties that gave great impetus to further studies on the effects of U.V. Gates[8] showed that when the bactericidal effectiveness of different wavelengths of U.V. is judged on the basis of dosage, an action spectrum is obtained, which relates the relative efficiency of the different wavelengths in killing bacteria. Hertel's tentative conclusion based on a small number of wavelengths was thus superseded. Gates' work was followed by many studies purporting to determine action spectra for various effects of U.V. on cells[9].

Gates also demonstrated that absorption of U.V. by bacteria does not correspond with the bactericidal action spectrum[10]. He searched for U.V.-absorbing substances in the cell which might have an absorption spectrum resembling the bactericidal action spectrum. This he found in the absorption by mixtures of purines and pyrimidines or substances containing these; for example, the nucleotides, nucleic acids and their derivatives[11]. He therefore suggested that effects of U.V. on nucleic acids underlie the action of U.V. radiations on cells

Meantime, discovery of other effects of U.V. were made, one of the most interesting

References p. 275

being the production of mutations, a field particularly actively investigated by Hollaender and his collaborators[12]. They showed that the action spectrum for induction of mutations resembled that for the bactericidal effect[13]. Other effects such as retardation of cell division under appropriate conditions show a similar action spectrum[9].

Not all effects of U.V. have an action spectrum like the bactericidal effect. Thus immobilization of ciliary activity in protozoa, sensitization of protoplasm to heat, etc. have an action spectrum much more closely resembling the absorption spectrum of a non-conjugated protein such as albumin or globulin[14,15]. Still other action spectra are found for initiation of parthenogenetic development in eggs and hemolysis of red blood cells[9].

Again, many interesting facts concerning the action of U.V. cells were discovered which defy cataloging in a brief introduction. One of the most interesting is the finding by Witkin[16,17] of a mutant strain of *Escherichia coli*, named B/r, which appeared among the survivors of strain B irradiated with a large dosage of U.V. This strain is also resistant to ionizing radiations. Another important set of studies are those on recovery of U.V.-treated cells and its dependence upon post-U.V. treatment[18], including heat[19].

It was at this time when interest in U.V. action upon cells was again lagging that our first speaker, Dr. Albert Kelner, made the dramatic discovery of the reversal of U.V. damage upon the spores of *Streptomyces griseus* by subsequent treatment with visible light; that is, *photoreactivation*. This discovery initiated a third period in the study of U.V. action upon cells. Many investigators, fascinated by the amazing fact of photoreactivation and hopeful that here they had a tool which promised to unravel the nature of radiation effects upon cells and give a further insight into life processes, left other studies to investigate this phenomenon. A large literature has accordingly accumulated and many interesting relationships have been uncovered.

This symposium was organized to present trends in the study of photoreactivation. To delimit the program the organizing committee asked that only work in progress be presented here. Consequently, if many individuals who have made important contributions to photoreactivation are omitted from the program, it is only because they have again turned to other problems. It is impossible here to review the entire field — this has been done in the perceptive review by Jagger[20], whom I wish to thank here for making many suggestions of individuals and topics for this symposium. It is our loss that he was unable to come to this meeting.

Our first speaker, Dr. Albert Kelner, will present the historical background to photoreactivation. Then follows a group of papers (Tageeva and Dubrov, Chessin and Biebl) concerned primarily with studies of photoreactivation in plants, a group of organisms not yet as completely investigated as animals and microorganisms. Dr. Biebl's paper, which explores chemical protection from U.V., is included primarily to induce others to try similar studies coupled with photoreactivation. The next paper (Ehret) concerns photoreactivation in protozoa. The following group of papers (Schiff and Epstein, Kleczkowski, Kaplan, Pittman, Hanawalt and Maaløe, and Fisher) represent analytical studies of specific phenomena as indicated by the titles. The papers on photoenzymatic repair of ultraviolet damage to DNA (Ruppert and Herriott, and Pakula) bring to a close the biological studies on photoreactivation. The last paper (Shugar) presents a possible model system to interpret on a molecular basis the way in which photoreactivation may work.

The discovery of photoenzymatic repair of U.V. damage to DNA[21] presents a unique opportunity to work at the molecular level on the process of photoreactivation. This finding may well initiate a fourth phase of U.V. studies, bringing them to the molecular level as has happened with so many other problems in cell physiology. Studies on the possible change in the nucleic acid molecules or their constituents discussed in our last paper represent another aspect of the attack at the molecular level. It is a pleasure to present to you the various papers in this symposium.

REFERENCES

1 A. Downes and T. P. Blunt, *Proc. Roy. Soc. London B*, 26 (1916) 488.
2 V. Bie, *Mit. Finsens med. Lysinst.*, 9 (1905) 75.
3 V. Bie, *Mit. Finsens med. Lysinst.*, 9 (1905) 5.
4 V. Bie, *Mit. Finsens med. Lysinst.*, 7 (1904) 65.
5 S. Bang, *Mit. Finsens med Lysinst.*, 9 (1905) 164.
6 E. Hertel, *Z. allgem. Physiol.*, 4 (1904) 1.
7 E. Hertel, *Z. allgem. Physiol.*, 5 (1905) 95.
8 F. L. Gates, *J. Gen. Physiol.*, 13 (1929) 231.
9 A. C. Giese, *Physiol. Zool.*, 18 (1945) 223.
10 F. L. Gates, *J. Gen. Physiol.*, 14 (1930) 31.
11 F. L. Gates, *Science*, 68 (1928) 478.
12 A. Hollaender and C. W. Emmons, *Cold Spring Harbor Symposia Quant. Biol.*, 11 (1946) 78.
13 A. Hollaender and C. W. Emmons, *Cold Spring Harbor Symposia Quant. Biol.*, 9 (1941) 170.
14 A. C. Giese and E. B. Crossman, *J. Gen Physiol.*, 29 (1945) 79.
15 A. C. Giese, *J. Cellular Comp. Physiol.*, 26 (1945) 47.
16 E. M. Witkin, *Proc. Natl. Acad Sci. U.S.*, 32 (1946) 59.
17 E. M. Witkin, *Genetics*, 32 (1947) 221.
18 A. Hollaender and W. D. Claus, *J Gen. Physiol.*, 19 (1936) 753.
19 E H. Anderson, *J. Bacteriol.*, 61 (1951) 389.
20 J. Jagger, *Bacteriol. Rev.*, 22 (1958) 99.
21 S H. Goodgal, C. S. Rupert and R. G. Herriott, in *Symposium on the Chemical Basis of Heredity* (W. S. McElroy and B. Glass, ed.), Johns Hopkins Press, Baltimore, 1957, p. 341.

HISTORICAL BACKGROUND TO THE STUDY OF PHOTOREACTIVATION

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As far back as 1904 Hertel[1] reported that visible light antagonized the ultraviolet-induced inhibition of protoplasmic streaming in *Elodea*. His experiments and other early ones were inconclusive and unconfirmed. We may better begin our history in 1933 with the experiments of Hausser and Oehmcke[2] on ultraviolet-induced browning of banana skin, and those of Whitaker[3] in 1942 on ultraviolet-induced inhibition of growth in *Fucus*. In both cases visible light reduced the ultraviolet effect.

Unfortunately even these studies made little impression on contemporary radiobiology; perhaps one reason was the interpretation of their data because of the nature of the material was also not clear cut. For example, visible light might have stimulated growth in *Fucus* by inducing photosynthesis, rather than by directly reversing ultraviolet-caused cellular damage.

Certainly in 1949 when photoreactivation of U.V.-inactivated microorganisms was discovered[4] there was little in current theory to lead one to predict photoreactivation. Many thought of high energy radiation affecting survival of the cell in an all-or-none fashion: a target was hit or not. If it was, that was the end of the cell.

There was by 1949 however increasing evidence that the effects of radiations might be influenced by post-irradiation conditions. Hollaender[5] had found that survival of U.V.-irradiated fungus spores was influenced by post-irradiation storage in liquid menstrua. Luria[6] had discovered multiplicity reactivation. Latarjet[7] and others had observed that in some cases survival of irradiated cells increased if they were kept cold for a period following irradiation.

When we first observed recovery of U.V.-irradiated *Streptomyces griseus* spores in our culture dishes we ascribed it to post-irradiation storage of the cells in the cold. When experiment did not support a "cold-reactivation" we searched for environmental factors which might be causing the overwhelming recovery. Among the reasons for testing light (besides its presence in the environment) was we thought it might act like cold in inhibiting germination of the spore and thus allow spontaneous recovery. (Incidentally, it did not inhibit germination).

In this paper we cannot attempt complete citation of the literature. Recent reviews such as Jagger's[8] do this well.

Photoreversal in bacteriophage emphasized that light was probably reversing U.V.-caused damage to DNA. Much of our knowledge on the kinetics of photoreactivation came from Dulbecco's fundamental experiments[9,10].

Many of the early works on photoreactivation dealt with effects of reactivating light

on survival or division of irradiated cells, and there was a suspicion that only physiological effects such as these were photoreactivable - - the genetic action of U.V. would not be so easily influenced by light. The demonstration that mutagenesis itself was as photoreversible[11-13] as other U.V. effects did its share in stimulating the fusion of radiation physiology and radiation genetics.

From 1949 through the middle 1950's were described many of the basic features of photoreactivation, which make it such a quantitatively predictable reaction: the kinetics, including the dose-reduction rule, the dark reaction, and action spectra for photoreactivation. However no undisputed mechanism was found, nor were the molecules concerned identified.

Photoreactivation was found in most major groups of organisms. Almost whatever U.V. did to the cell was photoreactivable, from loss of viability, inhibition of growth, to the inhibition of adaptive enzyme synthesis and mutation. That so many U.V. effects were reactivable was surprising. Were there many independent photoreactivation mechanisms, or were there only one or two key ones, which could explain all types of photoreactivations?

Many thought a likely key mechanism was that a U.V.-caused lesion to DNA was repaired by photoreactivation. There was the evidence from phage and from *Arbacia*[14]. Also von Borstel[15] and Giese[16] showed that in some cells, nuclear, but not cytoplasmic damage was photoreactivable. Moreover one of the first detectable effects of U.V. on *E. coli*, and one quite photoreversible was inhibition of DNA synthesis[17,18].

That a U.V.-caused lesion in DNA could be repaired by photoreactivation was finally proved by the remarkable discovery in 1957 by Goodgal, Rupert and Herriott[19] of *in vitro* photoreactivation of U.V.-irradiated transforming principle DNA. This discovery may mark the beginning of a new phase in photoreactivation research, with its promise that the biochemistry of photoreactivation will be clarified.

By 1957 the best established mechanism was that light (together with the dark reaction) repaired damage to DNA, and most photoreactivations could be explained on this basis. This cannot be the only mechanism, for photoreactivation has been described in enucleated amoebae[20], and in nerve cell cytoplasm[21]. These cases have a mechanism not involving DNA.

The DNA theory, does not explain well photoreactivation of damage (not necessarily the *primary* damage) to cytoplasmic elements, such as respiratory elements in yeasts[22], or chloroplasts in *Euglena*[23], or in RNA plant viruses[24]. It is noteworthy that in both respiratory elements in yeasts, and chloroplasts we have selfreplicating bodies, with genetic functions. Therefore a more accurate generalization (without excluding possibility of other mechanisms) which at present most of the data fits, is that it is U.V.-induced lesions to self-replicating elements, whether in the nucleus or cytoplasm, which are photoreactivable.

In closing, we ask whether such a widespread phenomenon, found in most of the living world, would exist unless it either had survival value in the past, or the same reactions (causing repair to genetic elements) had some counterpart in non-irradiated cells. Photoreactivation may be one of several mechanisms whose function is to maintain the stability of the genetic structure of the cell — a stability essential to life as we know it. The discovery of something homologous to photoreactivation in the normal cell, and understanding of its role in evolution would be a fitting part of research in photoreactivation.

REFERENCES

1 E. Hertel, *Z. allgem. Physiol.*, 4 (1904) 1.
2 K. W. Hausser and H. V. Oehmçke, *Strahlentherapie*, 48 (1933) 223.
3 D. M. Whitaker, *J. Gen. Physiol.*, 25 (1941-42) 391.
4 A. Kelner, *Proc. Natl. Acad. Sci. U. S.*, 35 (1949) 73.
5 A. Hollaender and C. W. Emmons, *Cold Spring Harbor Symp. Quant. Biol.*, 9 (1941) 179.
6 S. E. Luria, *Proc. Natl. Acad. Sci. U. S.*, 33 (1947) 253.
7 R. Latarjet, *Compt. rend.*, 217 (1943) 186.
8 J. Jagger, *Bacteriol. Rev.*, 22 (1958) 99.
9 R. Dulbecco, *Nature*, 163 (1949) 949.
10 R. Dulbecco, *J. Bacteriol.*, 59 (1950) 329.
11 A. Kelner, *J. Bacteriol.*, 58 (1949) 511.
12 A. Novick and L. Szilard, *Proc. Natl. Acad. Sci. U. S.*, 35 (1949) 591.
13 H. B. Newcombe and H. A. Whitehead, *J. Bacteriol.*, 61 (1951) 243.
14 H. F. Blum, J. C. Robinson and G. M. Loos, *J. Gen. Physiol.*, 35 (1951) 323.
15 R. C. von Borstel and S. Wolff, *Proc. Natl. Acad. Sci. U. S.*, 41 (1955) 1004.
16 C. L. Brandt and A. C. Giese, *J Gen. Physiol.*, 39 (1956) 735.
17 A. Kelner, *J. Bacteriol.*, 65 (1953) 252.
18 D. Kanazir and M. Errera, *Cold Spring Harb. Symp. Quant. Biol.*, 21 (1956) 19.
19 S. H. Goodgal, C. S. Rupert and R. M. Herriott, in *The Chemical Basis of Heredity*, Baltimore, 1957, p. 341.
20 Y. Skreb and M. Errera, *Exptl. Cell Research*, 12 (1957) 649
21 S. Pierce and A. C. Giese, *J. Cellular Comp. Physiol.*, 49 (1957) 303.
22 D. Pittman and P. R. Pedigo, *Exptl. Cellular Research*, 17 (1959) 359.
23 H. Lyman, H. T. Epstein and J. Schiff, *J. Protozool.*, 6 (1959) 264.
24 F. C. Bawden and A. Kleczkowski, *Nature*, 183 (1959) 503.

PHOTOREACTIVATION IN VIRUSES AND PLANTS

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As is seen in the latest review of Dulbecco[1], photoreactivation in plants has been studied insufficiently. In order to study the problem of photoreactivation in plant cells we used resting cells of the internal epidermis of onion scales *(Allium cepa)*. In our experiments a quartz-mercury SVD-120A lamp of superhigh pressure was used as the source of radiation. The energy distribution in this lamp spectrum is the following: visible area — 43%, UV spectrum 320-380 — 21%; 280-320 — 19%, 200-280 — 17% (Fig. 1). Evaluation of the amount of UV-radiation was carried out with the help of an Ultraviolmeter UV-I, developed in our Institute under the direction of Professor M. V. Sokolov.

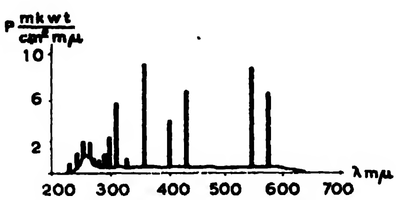

Fig. 1. The energy distribution in a quartz-mercury lamp of superhigh pressure, 120 A.

The UV radiation doses used in our experiments fell within the limits $0.5 \cdot 10^6$–$1.5 \cdot 10^7$ erg/cm², given within 6 to 180 sec of irradiation time without heating the epidermic cells. Before investigating photoreactivation we have studied the change of the functional state in epidermis cells after UV irradiation and the shifts in physico-chemical indices which define cellular viability. We have studied in detail the changes of pH in nucleus and cytoplasm, changes of redox conditions (rH_2), isoelectric points (I.E.P.), viscosity, cytoplasmic movement, plasmolysis, and sorptive abilities of cells. As the result of these investigations the threshold doses of UV radiation which cause profound irreversible changes in the functional state of the cell were determined immediately after irradiation. In Fig. 2 the threshold doses of UV radiation for various effects on plant cells are represented graphically. Cytoplasmic movement shows that the greatest sensitivity to UV radiation and movement ceases completely after a dosage of $2.0 \cdot 10^6$ erg/cm² (irradiation time 25 sec). The I.E.P. of cell colloids was most resistant to UV radiation: sharp changes in I.E.P. of nucleus and cytoplasm were observed only after a dose of $8.0 \cdot 10^6$ erg/cm² (irradiation time 100 sec). Plasmolysis and sorptive abilities proved to be very sensitive to the effect of UV radiation.

Therefore principal attention in our studies on photoreactivation in plant cells was

References p. 281

paid to investigation of restoration of movement, electrocolloidal, sorptive, and plasmolytic properties. Photoreactivation was realized by means of illumination of the irradiated epidermis sections with visible light for 15 min. The photoreactivation dose measured by Janishevsky's technique constituted 10^8 erg/cm^2. As our investi-

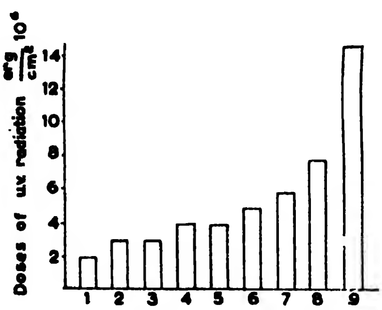

Fig. 2. The threshold doses of UV radiation for different physico-chemical indices and functional state in epidermal cells of *Allium cepa*. (*1*) Cytoplasmic movement; (*2*) redox conditions; (*3*) pH of nucleus; (*4*) viscosity of cytoplasm; (*5*) sorptive abilities of cells; (*6*) pH of cytoplasm; (*7*) plasmolysis; (*8*) pH IEP of cytoplasm; (*9*) pH of vacuole cape containing anthocyans.

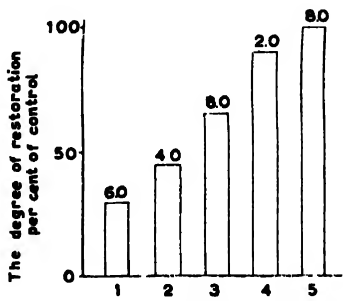

Fig. 3. The degree of photoreactivation of different cell functions in epidermal cells of *Allium cepa*. (*1*) Plasmolysis; (*2*) sorption abilities of cells; (*3*) pH IEP of cytoplasm; (*4*) cytoplasmic movement; (*5*) pH IEP of nucleus. The numbers above the columns show the threshold doses of UV radiation.

gation demonstrated, various degrees of photoreactivation are observed in physico-chemical factors and properties which reflect the functional state of the irradiated somatic cells. This statement is illustrated by Fig. 3 where the degree of restoration of the various properties studied is represented graphically. The greatest degree of restoration is found for cytoplasm movement and the I.E.P. of the nucleus and cytoplasm. But, restoration of these factors occurs at different threshold doses. Cytoplasmic movement is very sensitive to UV radiation, decreasing rapidly; at the same time it can be restored to a high degree by photoreactivation. This circumstance enables to understand better both the photoreactivation mechanism and the mechanism of primary UV radiation effects. It has been shown that the dose that brings about complete cessation of cytoplasmic movement ($2.0 \cdot 10^6$ erg/cm^2) produces serious changes in morphological properties of cell mitochondria, a finding in agreement with a report in the literature[2]. We have shown a distinct correlation between the complete cessation of cytoplasm movement and changes in morphological properties of mitochondria. This leads us to suppose that both phenomena are closely connected and reflect a primary cell reaction to UV radiation. We think that such an interdependence between cytoplasm movement and mitochondrial state can be expressed, in particular, by the fact, that the latter provide energy-supplying material necessary for the process of movement, for example — ATP. Support for this hypothesis on the primary mechanism of UV radiation comes from the finding that cytoplasm movement stopped by UV radiation can be restored to the normal level by addition of ATP in optimal concentration of 10^{-3} *M*. This circumstance leads us to believe that the disturbance of oxidative phosphoryiation is the cause of UV damage to somatic cells. Similar views have been expressed by other investigators (Beyer[3],

Jost and Robson[4]). On this basis the mechanism of photoreactivation in this case can be explained as the restoration of oxidative phosphorylation. Photoreactivation of enzymatic systems damaged by UV has already been postulated as the mechanism of photoreactivation (Errera[5]).

The action spectrum of photoreactivation, defined with glass light filters, by the sorption of fluorochrome acridin-orange within vacuoles of cells shows a maximum in the region 420 mμ; this is an indication of the possible participation of a flavine system as an acceptor of reactivation light.

Plant photoreactivation has ecological importance (Giese[6]). This can be illustrated by the papers, which point to the great formative role of UV rays and their quantitative correlation in the whole solar spectrum (Brodführer[7]). In some investigations the addition of short-wave irradiation from artificial sources of UV radiation at the background of the intensive radiation conditions in high mountains leads to the intensive growth and development of plants, in experiments of Professor Gurskii (personal communication, 1960).

REFERENCES

1 R. Dulbecco, *Radiation Biology*, 2 (1955) 455.
2 R. Biebl and W. Url, *Protoplasma*, 49 (3) (1958) 329.
3 R. Beyer, *Rad. Research*, 2 (1) (1958) 92.
4 H. Jost and H. Robson, *Biol. Bull*, 116 (3) (1959) 198.
5 M. Errera, *Progress in Biophysics*, 3 (1953) 88.
6 A. Giese, *Cell Physiology*, Chapter 4, 1957, p. 73.
7 U. Brodführer, *Planta*, 15 (1–2) (1955) 1.

A PHOTOREVERSIBLE EFFECT OF ULTRAVIOLET LIGHT ON CHLOROPLASTS

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In connection with experiments on the mechanism of photoreactivation (PR) of ultraviolet light (UV) — irradiated bean leaves, I have observed the apparent photoreversible disappearance of the chloroplasts in the guard cells of epidermis stripped from such leaves twenty-four hours after dark incubation[1]. The use of phase optics gives the same results as ordinary light. Starch also disappears from such plastids, which may partly account for the results.

Unexpectedly, even where leaves are given 10 × the minimal dose for plastid "disappearance", a high percentage of those few stomata which are still open function osmotically, as indicated by reversible plasmolysis deplasmolysis.

Recently, I had the opportunity to study irradiated plastids by means of fluorescence microscopy in Dr. S. Wildman's laboratory. Surprisingly, where no guard cell plastids could be observed by ordinary light or phase optics in irradiated leaves, discrete spots with the characteristic red fluorescence of chlorophyll could be seen easily when viewed in the fluorescence microscope. At higher UV doses, however, even these fluorescent spots disappear.

Apparently, there is some loss of plastid structure as a result of UV treatment (2537 Å) as indicated by observations with ordinary light or phase microscopy. However, the results of fluorescence microscopy show that the chlorophyll-binding skeleton (grana?) is more resistant to UV than some other parts of the plastid.

Schiff *et al.*[2], have demonstrated that at low doses of UV, plastid development in *Euglena* could be suppressed without interfering with subsequent cell division, thus giving rise to chlorophyll-free clones. At higher doses cell death results, although plastids apparently do not disappear if they are already present.

We have no concrete evidence for such a difference in UV sensitivity between plastid formation and cell viability, since our experimental material consisted of cells incapable of division. However, there is some indication that plastids are more sensitive to damage by UV than are some guard cells to loss of function.

REFERENCES

[1] L. Blakeley and M. Chessin, *Science*, 130 (1959) 500.
[2] J. Schiff, Harvard Lyman and H. T. Epstein, *J. Protozool.*, 6 (3) (1959) 264.

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PROTECTION AGAINST THE EFFECT OF SHORT-WAVE UV-RAYS ON PLANT CELLS

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The search for chemical substances to act as protective substances against the effects of ionizing rays (α-, β-, γ- and X-rays) today constitutes an important field of study in radiobiological research. This is due not only to the expected practical importance of these investigations but particularly to the resulting theoretical conclusions on the primary radiation effects in the protoplasm. Summaries on this subject have been given, amongst others, by Hollaender *et al.*[1], Bacq and Alexander[2], Langendorff[3], Pany[4] and Fritz-Niggli[5].

There is general agreement that these protective substances cannot influence the "hits" themselves, but can only affect the chemical products formed as a consequence of irradiation, as they appear in the aqueous phase of the protoplasm in the form of free radicles, peroxides, and ions. Although the active mechanism of the substances which protect from chemical radiation has not yet been elucidated in detail, there is no doubt that their effect is ultimately due to the neutralization of the chemically highly active ionizing products which arise at the moment of irradiation and which endanger the normal cell metabolism.

On the other hand, the fact that plant cells can be protected by means of such chemical substances to a considerable degree against the lethal effects of the short-wave ultraviolet rays of an ordinary quartz analysis lamp, shows that the short-wave ultraviolet rays lead to similar disintegration products in the protoplasm which are similar to those caused by ionizing rays in the narrower sense of the term (*cf.* Livingston[6]).

So far we have been working primarily with thiourea (Biebl[7]; Biebl, Url and Janecek[8]) and have been using for objects the inner and outer epidermata of scales of *Allium cepa*, as well as leaf epidermata of red cabbage (*Brassica oleracea*) and *Rhoeo discolor*.

The slices were placed for irradiation on moist filter paper, and were half-covered by means of a Schott WG5 filter-glass, through which only rays of a length exceeding *ca.* 310 mμ can pass. As the filter-screened slice halves did not show any damage even after the longest irradiation times, the changes occurring in the fully irradiated halves may be attributed to the action of ultraviolet rays shorter than 310 mμ. Observations were made in all cases 24 hours after irradiation.

A comparison of the effects of pretreatment of one onion inner epiderm for

* Research carried out with the support of the International Atomic Energy Agency. The comprehensive publication will appear in German in *Protoplasma*, 53 [3] (1961).

References p. 286

30 min with 0.1 *M* thiourea and of others with various other osmotically active substances such as urea, glucose, KCl, $CaCl_2$, or glycerine showed that thiourea is the only one of all these substances which possesses the protective effect. The lethal irradiation duration is usually extended by two- to threefold after treatment with 0.1 *M* thiourea, compared with the lethal duration in slices subjected to irradiation without any pretreatment.

A comparison of the pictures of un-pretreated and pretreated *inner epidermata of onion scales* shows that 24 h after 1 min of UV irradiation from a distance of 23 cm, only dead cells with detached, coagulated protoplasts were present in the un-pretreated slice, whereas the cells of the slice subjected to 30 min pretreatment with 0.1 *M* thiourea were all alive and, in a hypertonic glucose solution, showed fine, biconvexly rounded plasmolysis forms. In *red cabbage* leaf epidermis, the death of cells was clearly recognizable by the flowing out of the cell sap coloured red by anthocyan. After $2^1/_2$ min of UV irradiation the un-pretreated cells, irradiated on water, were dead and discoloured. The cells pretreated with thiourea, however, were all alive.

In order to determine the necessary *duration of pretreatment*, slices of onion inner epidermis were placed in 0.1 *M* thiourea 30, 15, 10 and 5 min before irradiation, and were subsequently irradiated on thiourea-soaked filter paper. The protective effect after 5 min pretreatment was only slightly less than that after 30 min pretreatment. Thus thiourea must have entered the cell in sufficient quantities very quickly.

A further experiment, in which slices pretreated at different durations in thiourea were, immediately before irradiation, replaced in water for 5 min and then irradiated on water-soaked filter paper showed that thiourea does not enter into a firm bond with any part of the cell. It was only in the slices pretreated for 30 min that a very slight protective effect could still be noticed. The effect of 5–15 min pretreatment, however, was completely removed by placing in water for 5 min.

In further tests, slices were irradiated without any pretreatment, only on thiourea-soaked filter paper, and in these cases, a noticeable protective effect was again achieved. It must be particularly emphasized that the upper sides of the slices were always blotted with dry filter paper before irradiation, so that during irradiation they were never covered by a liquid layer.

Finally, to facilitate a comparison with the slices pretreated for 30 min and consequently well protected, slices were irradiated without any pretreatment on water-soaked filter-paper, and were, immediately after irradiation or only after resting in water for different lengths of time, placed for 30 min in thiourea. In none of these cases could a definite protective effect be achieved.

The great role played by the *quantity* of thiourea present in the cell at the time of irradiation is illustrated by the following experiment. When slices of the inner epidermis of onion scale are placed in 0.001–0.5 *M* thiourea at equal pretreatment times of 30 min, and are afterwards irradiated on filter paper soaked with solutions of the same concentrations, the limit of the just noticeable protection is found at 0.05 *M* thiourea. 0.001 and 0.01 *M* thiourea are uneffective. With rising concentrations of thiourea the protective effect increases. A pretreatment in 0.5 *M* thiourea once more increases the protective effect of 0.1 *M* by the fourfold (Table I).

The protective effect of thiourea against the lethal effect of short-wave UV rays can thus be considered as proved.

Among the most sensitive, regularly observable reactions of the protoplasm of the

TABLE I

Allium cepa, INNER-EPIDERMIS OF ONION SCALE. DEPENDENCE OF RADIATION PROTECTION ON THE CONCENTRATION OF THIOUREA

Pretreatment (30 min)	Duration of UV-irradiation (min)																		
	1/4	1/2	3/4	1	1½	2	2½	3	3½	4	4½	5	5½	6	6½	7	7½	8	9
Control, H_2O	l	l	l	¦	+	¦	¦	¦	¦	¦									
0.001 *M* thiourea	l	l	l	:	¦	¦	+	¦	¦	+									
0.005 *M* thiourea	l	l	l	:	¦	¦	¦	¦	¦	¦									
0.01 *M* thiourea	l	l	l	¦	+	¦	:	+	¦	+									
0.05 *M* thiourea	l	l	l	l	l	¦	+	¦	¦	+									
0.1 *M* thiourea	l	l	l	l	l	l	¦	¦	¦	¦	+	¦	+	+	+	+	+	+	+
0.2 *M* thiourea	l	l	l	l	l	l	l	l	l	¦	¦	¦	¦	+	+	¦	+	¦	+
0.3 *M* thiourea	l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	+	+	¦	+
0.4 *M* thiourea	l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	+	+
0.5 *M* thiourea	l	¦	l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	l	¦

(l = living, ¦ = slightly dead, + = dead)

inner epidermis of onion scales against considerably sublethal UV irradiations (15 sec) is the *increase in water permeability* (Biebl[9,10], Toth[11], Biebl and Url[12]). It expresses itself in a faster onset of plasmolysis in the fully irradiated slice half as compared with the WG_5-screened control half. Together with this, there occurs, as a consequence of irradiation, a *decrease in viscosity*, which is shown in a faster and finer rounding of the fully irradiated protoplasts in plasmolysis.

The outer epidermis of onions, on the other hand, shows, as a result of sublethal irradiation, a considerable *blocking of glycerine permeability*. When outer epidermata of an onion scale half-screened for 3 min (lethal dose with 5 min irradiation) by a WG_5 filter are placed in a 1.0 *M* glycerine solution immediately after irradiation or 24 h thereafter, there at first occurs a normal plasmolysis in both cases. After some time, however, plasmolysis recedes again in the screened control half due to gradual glycerine permeation, whereas in the fully irradiated half it is retained due to the permeability blocking caused by irradiation.

It could thus be expected that these plasma properties should yield the most sensitive test for the effect of thiourea over the sublethal irradiation range. We were much surprised to find that *these radiation-conditioned permeability and viscosity changes are not influenced in any way by thiourea*. As in the slices irradiated on water without any pretreatment, the inner epidermata of onion scales in which the lethal dose for cells had been raised by three- to fourfold or beyond by means of thiourea pretreatment, showed after 15 sec irradiation and after immersion in a hypertonic solution, an earlier onset of plasmolysis and a finer rounding of the protoplasts than could be observed in the halves of the same slices that had been screened during irradiation by the WG_5 filter. And the outer epidermata of the onion scale irradiated with sublethal doses exhibited after pretreatment with thiourea the same blocking of glycerine permeability as could be seen in the un-pretreated controls.

References p. 286

It seems that there is a difference between the wave-lengths of the UV-radiation, which cause changes in permeability and viscosity of the protoplasm and the ones causing the death of cells.

REFERENCES

[1] A. Hollaender, C. C. Congdon, D. G. Doherty, T. Makinodan and A. C. Upton, *Proc. Intern. Conf. Peaceful Uses Atomic Energy, Geneva 1958*, 23 (1958) 3.
[2] Z. M. Bacq and P. Alexander, *Grundlagen der Strahlenbiologie*, G. Thieme, Stuttgart, 1958.
[3] H. Langendorff, *Atomkernenergie*, 3 (1958) 438.
[4] I. Paný, *Wien. klin. Wochschr.*, 69 (1957) 605, 621.
[5] H. Fritz-Niggli, *Strahlenbiologie*, G. Thieme, Stuttgart, 1959.
[6] R. Livingston, in: A. Hollaender (Editor), *Radiation Biology*, Vol. 2, *Ultraviolet and Related Radiations*, McGraw-Hill, New York, 1955, p. 1.
[7] R. Biebl, *Anz. Österr. Akad. Wiss.*, No. 2 (1960) 24.
[8] R. Biebl, W. Url and G. Janecek, *Protoplasma*, 53 (1961) 320.
[9] R. Biebl, *Protoplasma*, 36 (1942) 491.
[10] R. Biebl, *Protoplasma*, 37 (1942) 1.
[11] A. Toth, *Österr. Botan. Z.*, 96 (1949) 161.
[12] R. Biebl and W. Url, *Protoplasma*, 49 (1958) 329.

NOTE ADDED IN PROOF

The valuable suggestions arising from the discussion have led to further, complementary experiments. This is a summary of the results:

(*1*) Experiments in which quartz cuvettes filled with various thiourea concentrations were used as filters showed that concentrations corresponding to a 0.1 *M* thiourea layer of 5 μ or 10 μ thickness respectively protect the underlying epidermis slices of *Allium cepa* in a similar way as does a 30-min pretreatment with this solution.

(*2*) This seems to prove that the physical absorption of UV-rays in the thiourea contained in the cell, does in fact constitute the essential factor of the protection observed after thiourea pretreatment.

(*3*) A quartz cuvette, filled with 2% acetic acid of 1 cm thickness, still protects the underlying epidermis slices from the effect of the UV-radiation to a considerable extent. This proves that the UV-source used emits also harmful rays shorter than 230 mμ.

(*4*) In this short-wave UV-range, below 230 mμ, the possibility of increased formation of ions, radicles and peroxides exists. Therefore it may be presumed that here a chemical protection develops.

(*5*) However, it has not been possible to establish with certainty, whether or not in our experiments, the thiourea pretreatment also gave a slight chemical protection. The essential protective effect, as shown in Table I, is undoubtedly due to the physical absorption of the short-wave UV-rays by the thiourea mainly contained, possibly even accumulated, in the cell wall.

(*6*) The experiments will be continued with other substances.

PHOTOREACTIVATION OF ULTRAVIOLET-INDUCED CONJUGATION DELAY IN *PARAMECIUM**

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The criterion of damage and repair in many *in vivo* studies of photoreactivation is usually some measure of cell viability or of fission probability that involves nuclear mitoses. Two measures not involving nuclear mitoses have been made in *Paramecium bursaria*. The first, clearly not involving DNA replication, is that of U.V. induction and photoreactivation of induction of phase shift of the circadian rhythm for mating capacity. From experiments reported elsewhere, this effect can be correlated with RNA or with nucleotide-coenzyme syntheses which continue rhythmically even in non dividing cell populations[1].

The second measure, also in non-dividing cells, is that of conjugation delay. During conjugation, two meiotic divisions and one equational division of each parental micronucleus result in the production of a migratory and a stationary pronucleus within each parental cell. A reciprocal exchange between migratory pronuclei occurs, and is followed by the formation of a zygotic nucleus in each cell. About twenty-four hours after mating the cells separate to form new clones. Far-ultraviolet irradiation induces conjugation delay; this is dose-dependent, and at high doses some cells die while still fused.

Sensitivity to ultraviolet (254 mμ) appears to increase slightly until late prophase of the first meiotic division, after which it decreases significantly. Conjugation delay, therefore, correlates closely with inhibition of meiosis, and is probably caused by damage to this mechanism. This damage is photoreactivable with a dose reduction[2] of about 40%.

The magnitude of photoreactivation was not diminished when reactivating light was applied within 2.5 h after U.V., *i.e.*, up to the time of early prophase. Photorecovery was decidedly less when light was applied 4.6 h after ultraviolet treatment, during crescent to late prophase. However, instead of diminishing further, the magnitude of photoreactivation showed a slight increase after prophase, when the control population was in metaphase-telephase, 6.5 h after U.V. In general, the most photoreactivable stage is preprophase, and photoreactivation appears consistently least effective between early and late prophase[3].

The action spectrum for the photoreactivation of conjugation delay has been measured in conjugating cells in which inactivating and "reactivating" treatments

* This work was performed under the auspices of the U.S. Atomic Energy Commission.

References p. 288

both were applied before meiotic prophase. A high irradiance grating spectrograph yielding a linear dispersion of 1 Å per mm on the focal curve over the range from 2200 Å–8000 Å was employed. The reduction of conjugation delay and of the probability of death while fused were measured separately as a function of wavelength (Fig. 1). Each measure revealed (*1*) a single broad peak of spectral efficiency at 420 mμ, (*2*) enhancement of the deleterious effects of far U.V. by wavelengths shorter than 340 mμ, and (*3*) in the induction of half-maximum effects by either 254 mμ (delay) or 420 mμ (reactivation) the ratio of number incident quanta at 420 mμ per number of incident quanta at 254 mμ is over 50 to 1.

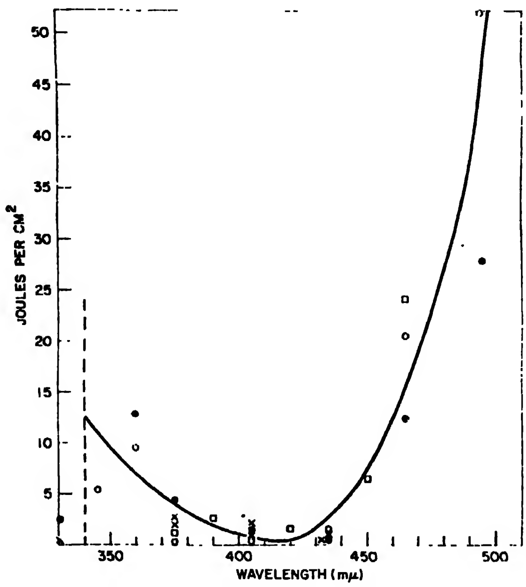

Fig. 1. Action spectrum for the photoreactivation of U.V.-induced conjugation delay and of death during conjugation in *Paramecium bursaria*.

Symbol	*U.V. dose*	*Phtr*
●, ○, □	3000 ergs/mm²	delay
✗	4500 ergs/mm²	death

While the photoreceptor is not uniquely characterizable, the action spectrum is grossly similar to that observed for photoreactivation in several other cells, and may represent ***Paramecium bursaria's*** own assemblage of chromophores in association with the Rupert Goodgal type of enzymes.

ACKNOWLEDGEMENTS

I am pleased to acknowledge the capable assistance of Paul Ellwanger and Gloria Kokaisl in the action spectrum determinations.

REFERENCES

[1] C. F. Ehret, *Cold Spring Harbor Symposia on Quantitative Biology*, 25 (1960) 149.
[2] C. F. Ehret and J. Fraembs, *Argonne Natl. Lab. Repts.*, 5518 (1956) 101.
[3] C. F. Ehret, A. Votava and L. Harrer, *Argonne Natl. Lab. Repts.*, 5655 (1956) 47.

ULTRAVIOLET INACTIVATION AND PHOTOREACTIVATION OF CHLOROPLAST DEVELOPMENT IN *EUGLENA*

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The ease with which chloroplast development in *Euglena* can be controlled by environmental conditions[1] led us to adopt this system to study the development and inheritance of a cell organelle, in this case the chloroplast, both as a means of understanding how the chloroplast develops as a functional unit, and to learn something of the processes controlling intracellular differentiation. Ultra-violet light has provided a useful tool in attacking these problems since it selectively inhibits chloroplast formation in the progeny of irradiated cells[2-5].

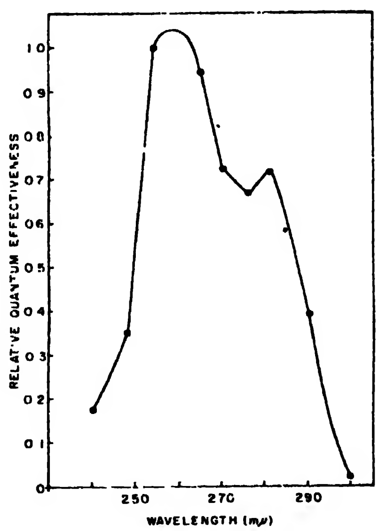

Fig. 1.

Our earlier studies on *Euglena* established the following[5]: *(1)* suspensions of light-grown cells (containing chloroplasts) which had been given sublethal doses of 2537 Å U.V. yielded colonies of chlorophyll and chloroplast-free cells ("albino" colonies) when plated under non-photoreactivating conditions; *(2)* the number of green colonies formed under these conditions approached zero at U.V. doses which did not affect cell viability; *(3)* when the same suspensions were plated under photoreactivating conditions, 100% photoreversal of the U.V. effect could be achieved; *(4)* suspensions of dark-grown cells (lacking chloroplasts or chlorophyll) when irradiated yielded the same pattern of inactivation and photoreactivation as green cells when plated under conditions which permit chloroplast formation; *(5)* cells obtained from albino colonies

References p. 292

produced by U.V. never revert to green and always yield colorless clones through extensive subculture in light or darkness; and *(6)* sectored colonies found on plates of irradiated cells were interpreted as arising from cells in which chloroplast formation had been partially inactivated by U.V. This suggested the presence of particles within the cells which might contain nucleic acid, which are heritable, and which control the formation of chloroplasts. Our more recent work[6] which we will describe now supports this hypothesis.

Our measurement of the action spectrum for the U.V. effect shows peaks in the vicinity of 260 and 280 mμ indicating that the chromophore might be a nucleoprotein (Fig. 1).

We have compared the U.V. doses required to yield a given number of albino colonies and find that the dark-grown cells are twice as sensitive as the light-grown cells. This finding admits of at least three interpretations: *(1)* That there is a change in the chemical nature of the chromophore during chloroplast development, *(2)* that the chloroplasts in the light-grown cells shade the chromophore containing particles or *(3)* that the chromophore-containing particles actually become incorporated into the developing chloroplast and are thereby shaded.

Our earlier data indicated that the photoreactivability of the U.V. effect decayed to zero over a five-day period. Experiments were carried out to determine the kinetics of decay. Dark-grown cells were given a dose of U.V. sufficient to yield close to 100% albino colonies when plated under non-photoreactivating conditions. These cells were

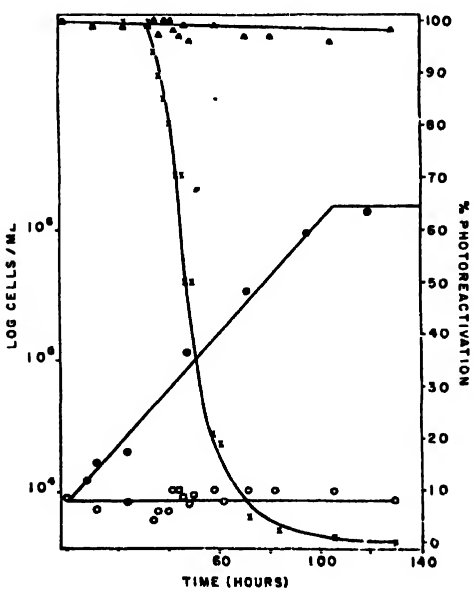

Fig. 2. $\triangle$ = % pr. in resting cells; $\times$ = % pr. in growing cells; $\bullet$ = growth of irradiated cells; $\circ$ = growth of irradiated cells in resting medium.

then inoculated into growing and resting medium in the dark. Aliquots were taken at various times thereafter and were plated under photoreactivating conditions. The results shown in Fig. 2 show that photoreactivability of the cells falls off rapidly when the cells are permitted to divide. Under non-dividing conditions, the cells remain completely photoreactivable indefinitely. It is possible that U.V. is acting to

prevent the replication of chloroplast-forming entities at cell division and that these entities are being distributed randomly among the progeny accounting for the decay in photoreactivability as the cells divide. We are currently undertaking a mathematical analysis of the data to see whether this hypothesis is justified.

To find out whether the light induction of chloroplast development in *Euglena* was related to photoreactivation (PR), an action spectrum was measured for PR of chloroplast formation. As seen in Fig. 3, the effectiveness spectrum has a broad peak in the near U.V., similar to PR in other systems such as *E. coli*[7,8]. Green or red light is completely ineffective. This separates PR clearly from the light induction of chloroplast

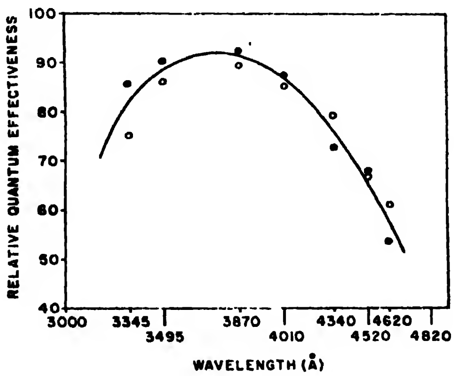

Fig. 3. ● expt. 1; ○ - expt. 2.

development since Nishimura[9] has shown that this action spectrum has peaks in the blue and red regions of the spectrum, so we conclude that the chromophores for PR and chloroplast induction are different.

The differences in these action spectra have enabled us to design experiments which further circumscribe the action of U.V. in this system since it is now possible to induce chloroplast formation in U.V.-treated cells without bringing about concommitant photoreactivation. If dark-grown cells which have been given a U.V. dose sufficient to yield almost 100% albino colonies when plated are exposed to red light, we find that the transformation of protochlorophyll to chlorophyll still occurs normally. This shows that U.V. does not act at this early stage of chloroplast formation. A second experiment carries this further. The irradiated dark-grown cells are inoculated

TABLE I

THE SPECIFIC EFFECT OF U.V. ON CHLOROPLAST REPLICATION

Irradiation	*Post U.V. lighting conditions*	*Observations of chloroplast development**	*Cells plated after chloroplast development*	
			PR cond.	*Non PR cond.*
No U.V.	PR light**	Normal	100% green***	100% green
U.V.	PR light	Normal	100% green	100% green
U.V.	Red (no PR) light	Normal	100% green	100% white
No U.V.	Red (no PR) light	Normal	100% green	100% green

* By fluorescence microscopy
** PR = photoreactivating light provided by daylight fluorescent tubes
*** Colonies

References p. 292

into resting medium and are exposed to red light. Chloroplast and chlorophyll production are followed by subjecting aliquots to fluorescence microscopy at various times after U.V. As may be seen from the summary in Table I, chloroplasts and chlorophyll develop normally in the U.V.-irradiated cells. When aliquots of these greened cells are plated under non-photoreactivating conditions, 100% albino colonies are obtained. The controls show that the cells are still capable of being photoreactivated at the end of the experimental period. This experiment clearly shows that the effect of U.V. is not on chloroplast development from precursors in the dark-grown cells, but that U.V. inhibits the replication of chloroplasts, or the sites that make them, at cell division.

On the basis of the above experiments, we infer that there are heritable nucleoprotein entities in the cells which control chloroplast development. The absence of lethality when chloroplast-forming ability is inactivated completely, the experiments on PR decay, the presence of sectored colonies on the plates and the difference in sensitivity of dark and light-grown cells all suggest to us that the sites involved are cytoplasmic.

We have attempted to find structures in dark-grown *Euglena* which serve as chloroplasts precursors in the hope that these might be identical with the U.V. sensitive sites mentioned above. Fluorescence microscopy[10] showed the presence of red-fluorescing particles in the dark-grown cells. These could be seen to enlarge when the cells were placed in the light and eventually developed into mature chloroplasts. This has been supported by our recent findings with electron microscopy which reveal precursor particles about 1 μ in diameter in the dark-grown cells. During development, these enlarge and develop a lamellated structure. The formation of lamellae appears to be linear with time, the time to make a lamella being of the order of 6 h. The first detectable photosynthetic oxygen evolution appears to be correlated with the appearance of the first lamella[11].

Our efforts in the future will be directed toward finding out whether the U.V.-sensitive entities and the structures found by fluorescence and electron microscopy are identical as well as correlating the steps in chloroplast formation with their physiological functions and seeking to understand the mechanisms controlling this type of cellular differentiation.

ACKNOWLEDGEMENT

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REFERENCES

1 S. Hutner and L. Provasoli, in A. Lwoff (editor), *The Protozoa*, Vol. 1, Academic Press, 1951.
2 E. Pringsheim, *Rev. Algologique (New Series)*, 4 (1958) 41.
3 M. DeDeken Grenson, *Arch. intern. physiol. et biochem.*, 67 (1959) 506.
4 J. Meyers, Personal Communication.
5 H. Lyman, H. T. Epstein and J. A. Schiff, *J. Protozool.*, 6 (1959) 264.
6 J. A. Schiff, H. Lyman and H. T. Epstein, *Proceedings, IXth International Botanical Congress*, 1959.
7 A. Kelner, *J. Gen. Physiol.*, 34 (1951) 835.
8 J. Jagger and L. Latarjet, *Ann. inst. Pasteur*, 91 (1956) 858.
9 M. Nishimura and H. Huzisige, *J. Biochem. Tokyo*, 46 (1959) 225.
10 H. Epstein, E. Boy de la Tour and J. A. Schiff, *Nature*, 185 (1960) 825.
11 A. Stern, J. A. Schiff and H. T. Epstein, *Unpublished work*.

PHOTOREACTIVATION IN VIRUSES AND PLANTS

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As infectivity of a virus seems to be a function of its nucleic acid, we assume that inactivation of infectivity of a virus by U.V. and its photoreactivation by visible light both result from effects of these radiations on the nucleic acid. Inactivation is a direct effect of radiation on the nucleic acid, whereas photoreactivation seems to be an indirect effect, radiation energy being first absorbed by some material that belongs to the host. Photoreactivation of viruses or of their isolated nucleic acids has so far been achieved only inside their hosts.

Most bacterial and plant viruses that have been tested so far, showed the phenomenon of photoreactivation, and only a few plant viruses did not. As far as I know, no animal virus has ever been tested.

The only virus whose inactivation by U.V. and that of its nucleic acid has been investigated quantitatively, is tobacco mosaic virus (TMV). It belongs to a few plant viruses whose inactivation by U.V. can not be reversed by photoreactivation, but inactivation of its isolated infectious nucleic acid can be partially reversed by photoreactivation (Bawden and Kleczkowski[1]).

The fact that becomes immediately obvious when a comparison is made between inactivation by U.V. of infectivity of intact TMV and of its isolated nucleic acid is that the nucleic acid inside the intact virus is very much more resistant to U.V. than it is when isolated (McLaren and Takahashi[2]; Bawden and Kleczkowski[1]). There is some protection of nucleic acid from U.V. inside the virus because of shading by protein, but this is relatively small, and what I am considering now is the fact that nucleic acid itself is very much more resistant to U.V. when it is a part of the virus than when it is isolated. When the isolated nucleic acid is irradiated by U.V., about half of absorbed radiation energy causes the kind of damage that is reversible by photoreactivation and the other half causes damage that is not reversible. When intact virus is irradiated, inactivation of nucleic acid by radiation energy absorbed by it progresses at a rate that is roughly only about 1/10th of that of free nucleic acid, and the kind of damage that is reversible by photoreactivation does not occur at all.

The probable reason for the greater resistance to U.V. of the nucleic acid when it is a part of the intact virus than when free, is that the bonding between the nucleic acid and the protein reinforces the structure of components of nucleic acid. The degree of protection of nucleic acid by protein may vary between different viruses and even between strains of the same virus, like for example the strains U1 and U2 of TMV (Siegel, Wildman and Ginosa[3]). The results obtained by Kassanis[4] suggest that the nucleic acid of a tobacco necrosis virus is protected very little or not at all by the protein component. These differences between viruses, or between strains of viruses,

References p. 295

can be explained by assuming differences in the nature of bonding between protein and nucleic acid.

The fact that in tobacco mosaic virus the bonding with protein completely protects nucleic acid from the kind of damage by U.V. that is reversible by photoreactivation, so that only irreversible kinds of damage can occur, explains why this virus is not photoreactivable after irradiation with U.V. It is quite possible that different nucleic acids may differ from one another intrinsically in the extent to which the damage by U.V. can be reversed by photoreactivation, but the way in which nucleic acids are bonded with protein in different viruses or in different other organisms may certainly influence the degree to which they can be photoreactivated after irradiation with U.V.

When inactivation of a virus by U.V. is investigated by irradiating free virus in controlled environment, usually quite consistent and reproducible results are obtained, and the only factor that makes results of some computations uncertain, is the uncertainty as regards proportions of absorbed and scattered radiation energy. When, however, inactivation of a virus is intended to be investigated inside the host plant, the problem becomes complicated not only by the possibility of U.V. affecting the host itself, but also by the possibility of protection of the virus from U.V. by the host plant. There was a tendency in the past among some plant virus workers simply to ignore these possibilities.

There is, first of all, the lethal effect of U.V. on plant cells. This can be reversed to some extent by photoreactivation (Bawden and Kleczkowski[5]; Tanada and Hendricks[6]; Chessin[9]), but there is no quantitative information available to show either the rate at which plant cells are killed or the extent to which this is reversed by exposure to visible light. The cell that has not been killed by U.V. or has been prevented by photoreactivation from dying; may, however, be altered in various ways and one of them may be a change in response to infection by a virus. There may be many different ways in which response of a cell to virus infection can be altered, but the only alteration in plants that has been studied so far concerns the *capacity* to support virus multiplication to the extent of forming local lesions. The effect of U.V. on capacity was assessed from alterations in numbers of local lesions produced by inoculating with a standard inoculum after irradiating the plant. Hence there is no way of knowing whether irradiation affects capacity by influencing only the initiation of infection, only some later stages in the process, or both the initiation and the development of infection.

Susceptibility of capacity to U.V. varies very considerably not only between different species and varieties of plants, but between plants of the same variety and between leaves of the same plant, and it depends on such factors as the age of the plant, the season of the year, temperature and illumination for a short time before irradiation with U.V., etc. (Bawden and Kleczkowski[7]; Sinha[8]). It is not possible, therefore, to give any quantitative relationship between the amount of irradiation per unit area of leaf surface and the effect on capacity that would be generally applicable to any particular variety of plants.

Fig. 1 gives an example of the variation*. Numbers of local lesions formed on leaves of *Nicotiana glutinosa* inoculated with TMV after they had been irradiated with U.V., shown as percentages of those formed on non-irradiated control leaves, are plotted

* Slide shown during the Congress.

against the dose of irradiation. Experiment 1 was done in the summer and Experiment 2 in the winter. The lines marked *L* show the results obtained on the leaves that were exposed to daylight after irradiation with U.V. and the lines marked *D* show the results obtained on the leaves that were kept in darkness for 24 h after irradiation. Differences between the positions of corresponding points on lines *L* and *D* show the extent of photoreactivation. Obviously capacity of the plants raised in the summer was much more resistant to U.V. than that of the plants raised in the winter. It can also be seen that when photoreactivation did take place, capacity of the irradiated plants at first increased above the original level, and then fell down as the dose of irradiation increased.

In usual conditions photoreactivation of a plant's capacity takes a few hours to reach completion (Sinha[8]). With such a stable virus as TMV, full extent of photoreactivation of a plant's capacity can be demonstrated almost irrespective of whether the virus is inoculated when photoreactivation is already complete, or whether it is inoculated immediately after irradiating the plant with U.V. A virus particle can apparently survive in a site whose capacity to support virus multiplication is destroyed by U.V., and start multiplying when capacity of the site is restored by photoreactivation. On the other hand, when the plant is inoculated with free nucleic acid isolated from TMV, full extent of photoreactivation of the plant's capacity can only be shown if inoculation is done after photoreactivation has reached completion. Nucleic acid that can not immediately start multiplying because capacity of the sites is destroyed by U.V., apparently becomes inactive, and can not start multiplying when capacity of the sites is subsequently restored by photoreactivation (Bawden and Kleczkowski[7]). Behaviour of some other viruses or of their isolated infectious nucleic acids can be intermediate between the two extremes. The behaviour of red clover mottle virus (Sinha[8]) is an example. There is also some variation in the behaviour of each virus that seems to depend on the condition of the host plant.

REFERENCES

1 F. C. Bawden and A. Kleczkowski, *Nature*, 183 (1959) 503.
2 A. D. McLaren and W. N. Takahashi, *Radiation Research*, 6 (1957) 532.
3 A. Siegel, S. G. Wildman and W. Ginosa, *Nature*, 178 (1956) 1117.
4 B. Kassanis, *Virology*, 10 (1960) 353.
5 F. C. Bawden and A. Kleczkowski, *Nature*, 169 (1952) 90.
6 T. Tanada and S. B. Hendricks, *Am. J. Botany*, 40 (1953) 634.
7 F. C. Bawden and A. Kleczkowski, *Virology*, 10 (1960) 163.
8 R. C. Sinha, *Thesis*, University of London, 1960.
9 M. Chessin, *Ann. Appl. Biol.*, 46 (1958) 388.

PHOTOREVERSAL OF *c*-MUTATIONS INDUCED BY U.V. IN THE EXTRACELLULAR PHAGE KAPPA

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Mutations of the T-phages or λ of *B. coli* are induced by radiation only when the host bacteria are also irradiated (Harm[1]). Recently Krieg[2] demonstrated that rare mutations of the rII region appear after extracellular U.V.-irradiation of phage T4.

In the phage Kappa of *Serratia marcescens*, Ellmauer and Kaplan[3] observed up to about 1.5% clearplague (*c*-) mutations after U.V.-irradiation of only the extracellular phage particles. This temperate phage forms turbid plaques with a violet diffuse halo in the red layer of the indicator bacteria (strain *Serratia* HY). The mutant plaques are completely clear (*c*) or still a little turbid[1], probably due to a reduction of the lysogenizing power. The degree of clearness is a specific character of each mutant type; about 1/3 of all mutations are still more or less turbid. Other types of plaque-mutation, *e.g.* with a red, a pale or a sharp limited halo, are extremely rare after U.V. irradiation. Therefore it seems that the *c*-region of the genom is preferentially mutable by U.V. This view is supported by the observation that all of 12 isolated *c*-mutants, when investigated by U. Winkler with the mixed-infection test of Kaiser[4], proved to be functionally allelic, *i.e.* they are mutated in the same cistron. Recently, recombination between other loci were found by Wolf-Ellmauer (unpublished). Further support is given by the discovery by S. K. Bose (unpublished) that nitrous acid induces many other types of mutations besides the *c*-type.

The U.V.-dose curve of the *c*-mutations has the shape of a 2 hit function with a saturation level between 15–20 per 10^3 survivors. The inactivation of reproduction of the phage is a 1 hit process. This indicates that the two effects are due to different changes by U.V., very probably in the phage DNA. Since post-cultures of freshly induced *c*- or *l*- plaques show only the mutant type it seems probable that the 2 hits by U.V. leading to mutation have usually changed both strands of the DNA.

The probability of an inactivation hit is $2.7 \cdot 10^{-2}$/sec U.V. (U.V. intensity 20 erg/mm^2/sec); the probability of a single mutation hit is $2.8 \cdot 10^{-4}$, which is about 100 times smaller. If we assume that the sensitivity to U.V. hits is equally distributed along the DNA of the genom and that inactivation hits can occur efficiently in the whole genom then the target of *c*-mutations, being 100 times smaller, would agree with the size of a cistron derived from the observations of other authors (*e.g.* Harm[1]). This leads to the hypothesis that 2 U.V. lesions in the c-cistron, each of them in a base of one nucleotide-strand, would initiate a *c*-mutation.

We may ask now whether the change due to the 2 U.V. hits in the DNA is already

a perfect mutation or a premutative and still reversible state. This question could be answered by experimental posttreatments after U.V.-irradiation, especially by photoreversal. In bacteria it is known that the mutation process induced by U.V. (as well as killing) is reversible by posttreatment with visible light, heat or chemicals. Photoreversal of colour mutations in *Serratia* was studied intensively by Kaplan[5] and Kaplan and Gunkel[6]. In the case of intact cells 2 proposals to explain this photoreversal are possible:

(1) the U.V. is absorbed outside the gene, it acts *indirectly*, and the light interferes with a premutational step outside the genes, *e.g.* a changed metabolic reaction or a U.V.-induced "poison";

(2) the U.V.-hits occur *directly* in the DNA and change it to a "metastable" state which can be reversed by light. The receptor of the light may be some substance, *e.g.* a metabolite outside the gene, or it may be the DNA itself, *e.g.* a U.V.-activated state in it. It is not yet possible to decide which of these alternatives is correct (see *e.g.* Jagger[7]). Our previous finding that the s-mutations (not the killing) of *Serratia* strain K are reversed even by illumination of strongly dehydrated cells could mean that metabolism is not necessary for photoreversal (Kaplan and Kaplan[5]).

Photoreversal of mutations has now been found in the extracellular phage Kappa. Post-illumination of the free U.V.-irradiated phage at a constant temperature of 30° reduced the yield of *c*-mutations to about 2/3 or 1/2. Inactivation, on the other hand, was decreased only a few percent. Blue-violet light (8 Osram-photocopy-tubes HNP 90 in a distance of 5 cm; emission spectrum 320–600 mμ, maximum at 420 mμ) induced maximum reversal after a dosing-time of 10 min; white light (8 Osram daylight tubes HNT 90, emission between 380 and 800 mμ, maximum at 620 mμ) only after 60 min. The light-dose curve of the *c*-mutations initially falls approximately linearly to a minimum of about half the original value after 10 min of photocopy-light illumination; it then goes upward again, 60 min giving about the original value. When light was given 1/2 h after infection of cells by the U.V. treated phage, *i.e.* intracellularly, the mutation reversal was very small (13.9 to $10.7 \cdot 10^{-5}$) but the survival after 4.5 min U.V. increased strongly from $2.0 \cdot 10^{-3}$ to $1.9 \cdot 10^{-2}$. This shows that the mutation reversal is not improved by the intracellular conditions but that the photoreactivation of killing is. Thus metabolism seems not to be necessary for reversal but is perhaps for reactivation. The photoreversal of the free phage is not due to substances dissolved in the lysate fluid, because phages both in the raw lysate and after washing in buffered saline gave about the same reversal. We must conclude from these experiments that the mutative lesions induced in DNA of the free phage are at least partially reversible premutations. Probably the phage DNA is the U.V. receptor as well as photoreceptor.

The increase of the mutation yield at high light doses can have two causes: (1) The lesions "reversed" (incompletely) by the light could be re-converted to mutative lesions by further absorption of light quanta. (2) Certain lesions which do not give mutations under usual conditions could be changed by (several) light hits to mutative lesions.

The U.V.-dose curve with 10 min post-illumination of the free phage by photo-copy-light is concave with a power of 2 at the beginning similar to the dark U.V.-dose curve. A constant dose reduction does not appear to exist since this quotient falls systematically from 0.80 at 1.5 min U.V. to 0.55 at 7.5 min U.V. Rather, the mutation *percentage* seems to be reduced constantly by a factor of 0.72. This would mean that the light

does not independently cancel the 2 U.V. hit-effects (in this case the dose function would be $m = f(pD)$, where p is the reduction factor), but first reverses the joint result of both hits ($m = p \cdot f(D)$). It seems that there is a superposition of both U.V. hits in the premutation as was also indicated in the case of the s-mutations of *Serratia K* (Kaplan and Gunkel[6]). This is feasible if the 2 bases of the same pair of nucleotides in the DNA were changed by the 2 hits.

The photoreversible premutative state seems to be sensitive to unknown factors, because in 2 of 8 experiments with post-illumination the reversal was not marked with certainty although a very small photoreactivation was always observed. One could presume that the reversible premutative state in the phage DNA is similar to the reversible change of a double bond in the pyrimidine rings which Sinsheimer[8] supposed to be the cause of the reversible decrease of the absorption peak after U.V.-irradiation of cytosine or uracil. Because Sinsheimer[8] found the absorption change of these substances to be reversible by heat we performed post-treatment temperature experiments on the U.V.-irradiated phage. It was found that 30° was ineffective but 40° for 30 min reduced the mutations from 8.0 to $4.6 \cdot 10^{-3}$. After longer exposure the mutation percentage increased again. At higher temperatures the minimum due to reversal was only small and it was followed by a high peak exceeding the initial mutation percentage. It would appear that 40° is the optimum temperature for reversal and is similar in action to light. (In the photoreversal experiments the temperature of the phage suspension was much lower).

Experiments with post-treatment at pH 5, 7 and 10 did not reveal a significant influence of the pH on the *c*-mutations, though Sinsheimer[8] had found a strong reversal of the effect studied by him at low and high pH.

Early experiments with *Serratia* (Kaplan and Gunkel[6]) revealed reversal of the U.V.-induced *s*-mutations by post-treatment with halide ions, the ions in 0.25 *M* Na-salt solution enhancing reversal according to the series $Cl^- < Br^- < I^- < F^-$. Reversal of the U.V.-induced *c*-mutations in the phage Kappa was enhanced by the ions according to the same series (Park, unpubl.). Because in both cases the same order of effectiveness of the ions was found it appears very probable that in the phage as well as in the bacterial cells the ions act on the premutative lesions in the DNA, perhaps by a change of the degree of hydration of this genetic substance. Before this hypothesis on the chemical nature of the photoreversible state in the gene can be judged definitely further studies with U.V.-irradiated DNA, nucleotides etc. *in vitro* and comparisons with the mutations in phage and bacteria are necessary.

REFERENCES

1 W. Harm, in *Strahlenbiol. etc., Ergeb.* 1952–58, Verlag Thieme Stuttgart 1959, p. 69.
2 D. R. Krieg, *Virology*, 9 (1960) 215.
3 H. Ellmauer and R. W. Kaplan, *Naturwiss.*, 46 (1959) 150.
4 A. Kaiser, *Virology*, 3 (1957) 42.
5 R. W. Kaplan and Ch. Kaplan, *Exptl. Cell Research*, 11 (1956) 378.
6 R. W. Kaplan and W. Gunkel, *Arch. Mikrobiol.*, 35 (1960) 63.
7 J. Jagger, *Bacteriol. Rev.*, 22 (1958) 99.
8 R. L. Sinsheimer, *Radiation Research*, 6 (1957) 121; *Radiation Research*, 1 (1954) 505.
9 R. W. Kaplan, U. Winkler and H. Wolf-Ellmauer, *Nature*, 186 (1960) 330.

GENETICALLY CONTROLLED PHOTOREACTIVATION OF AN ULTRA-VIOLET-INDUCED EXTRACHROMOSOMAL MUTATION IN YEAST*

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Numerous investigations, dating from Kelner's physiological studies[1] on ultraviolet-inactivated and photoreactivated bacteria, may be cited in support of the hypothesis that photoreactivation (PR) mechanisms are under genetic control. These studies are discussed in detail elsewhere[2] and attention is focused here only on two recent lines of work which offer compelling evidence for this hypothesis. The first of these is the enzyme-dependent PR *in vitro* of ultraviolet-inactivated transforming factor of *Hemophilus*[3]. The enzyme required is present in *Saccharomyces* and *Escherichia coli*, both photoreactivable, but absent in *Hemophilus*, which is nonphotoreactivable. We may infer that the enzyme is under genetic control and plays a role in PR of the intact cell, although conceding that neither inference is supported by direct experimental evidence. The second line of work (discussed below) is the PR of an "extrachromosomal" mutation in yeast, in which photoreversal of the mutational process has been shown[4] to be under control of a single gene-pair showing simple dominance for the photoreactivable trait.

Gene-controlled photoreactivation of an extrachromosomal mutation in yeast

Respiration in yeast is under the control of a gene-pair (r+/r) showing simple dominance and a "self-replicating, extrachromosomal" factor[4,5]. For convenience the presence (absence) of the functional extrachromosomal factor is designated y+ (y). Wildtype yeast (r+ y+) carry both the dominant allele for respiration and a functional extrachromosomal factor; genic mutants (r y+), although respiration-deficient, transmit the extrachromosomal factor, while the respiration-deficient "petite" mutants (r+ y) lack the factor or carry it in a nonfunctional state.

When normal (r+ y+) and genic (r y+) stocks are irradiated with intermediate doses of ultraviolet, a large proportion of the survivors form colonies which carry the extrachromosomal defect (r+ y and r y, respectively). However, as shown in Table I and Fig. 1, only the mutation in normal stocks is photoreactivable. The mutation is also photoreactivable in homozygous dominant (r+/r+) and heterozygous (r+/r) diploids but nonphotoreactivable in the homozygous recessive (r/r) stock[4] (Table I). The heterozygote on sporulation produces two normal (r+ y+) and two genic (r y+) segregants; the mutation is photoreactivated in the former two

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References p. 304

TABLE I

PHOTOREACTIVATION OF THE EXTRACHROMOSOMAL MUTATION IN HAPLOID PARENTS, IN DIPLOIDS HOMOZYGOUS DOMINANT, HETEROZYGOUS, AND HOMOZYGOUS RECESSIVE FOR THE r GENE-PAIR AND OF ASCOSPORE SEGREGANTS OF ONE TETRAD OF THE HETEROZYGOTE[4]

	Cultures	*Mutant induced*	*% Mutants* Dose = 0		1,500 ergs/mm²	
			Dark	+ PR	Dark	+ PR
Haploids	8256 r+ y+	r+ y	0.7	1.9	36.1	15.1
	8282 r+ y+	r+ y	0.5	0.5	39.5	12.5
	8256.27 r y+	r y	1.6	1.9	36.0	41.0
	8282.3 r y+	r y	0.8	0.7	48.5	52.5
Diploids	8256 × 8282	r+ r+ y	1.4	1.0	26.9	8.6
	8256.27 × 8282	r+ r y	1.7	1.3	37.6	15.1
	8256.27 × 8282.3	r r y	1.2	2.7	45.2	47.5
Ascospore segregants of the heterozygote 8256.27 × 8282						
	1a r y+	r y	2.6	2.8	34.0	39.3
	1b r+ y+	r+ y	1.2	1.6	32.1	13.8
	1c r+ y+	r+ y	0.9	1.2	36.3	15.9
	1d r y+	r y	1.0	0.8	40.2	39.0

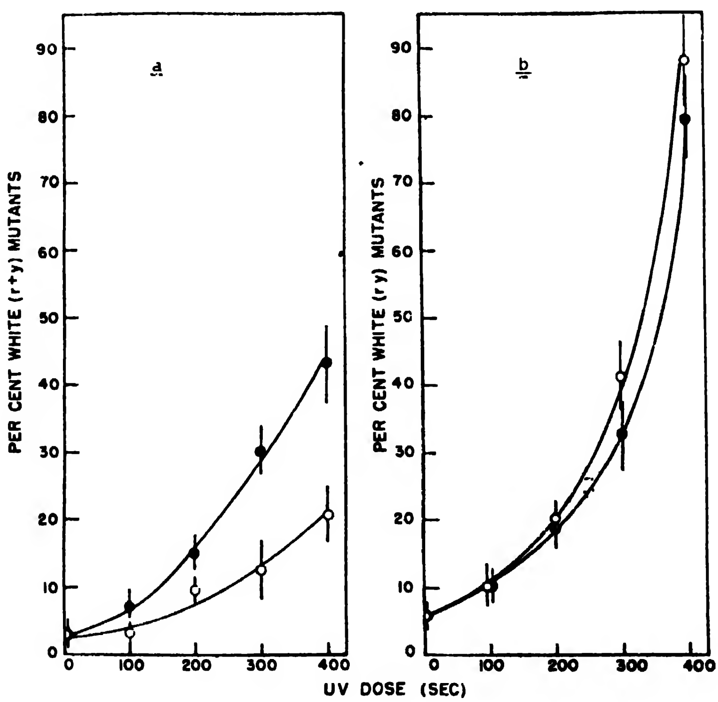

Fig. 1. a, Culture irradiated: 8256 (r+ y+), ● = dark, ○ = PR. b, Culture irradiated: 8256.27 (ry +), ● = dark, ○ = PR.

segregants but not in the latter two (Table I). Ultraviolet inactivation (killing), on the other hand, is photoreactivated to essentially the same degree in both the parents, the diploid, and the segregants[4], indicating that different steps are involved at some point in the photoreactivation of mutation and killing. PR dose-response curves for mutants

induced in normal and genic stocks subjected to $1.5 \cdot 10^3$ ergs/mm^2 are shown in Fig. 2. The frequency of mutants (r y) induced in the genic strain remains essentially the same with increasing dose of PR light, while the frequency of mutants (r+ y) induced in the normal stock decreases rapidly after a short lag to a saturation level of about one-half that of the initial frequency. From these studies we hypothesized

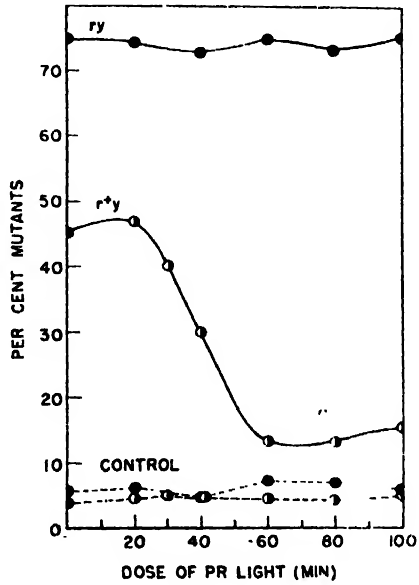

Fig. 2.

that r+ stocks carry a cellular component (either a chromophore or enzyme) necessary for photoreactivation of the extrachromosomal mutation. Preliminary studies with snail enzyme-treated r y+ stocks irradiated and photoreactivated in the presence of r+ y+ extracts are consistent with this hypothesis.

Ultraviolet-inactivation and photoreactivation of subcellular fractions containing the y+ factor

The above hypothesis is open to the objection that the self-replicating character of the y+ trait, although supported by adequate genetic data, is purely hypothetical. Recent studies[2,6] have shown, however, that subcellular fractions of r y+ stocks carry an active component capable of transforming r+ y stocks to wild type. Preparation of this active fraction and its sensitivity to ultraviolet radiation and subsequent PR are described below.

Haploid culture 8256.27 r y+ grown aerobically for 20 h in nutrient broth is harvested, washed three times in $M/15$ KH_2PO_4 buffer, packed (3 g wet weight) by centrifugation and homogenized with a glass homogenizer for 20 min (0°). The homogenate (90% broken cells) is suspended in buffer to 10 ml and centrifuged successively (*ca.* 20 times) at 700 rpm (3 min) to remove whole cells and "ghosts." The supernatant, containing cell fragments and particles, is spun at 12,000 *g* for 1 h (5°), the supernatant (inactive) discarded and the sediment suspended to 8 ml in buffer. The resulting

References p. 304

suspension (PF) contains large cell fragments and particles, exhibits no respiratory activity; most of the lipids and carbohydrates are retained in the supernatant[2]. Analysis of the 12,000 *g* residue and supernatant for protein and nucleic acids is shown in Table II. There is approximately twice the protein in the supernatant as in the residue. All of the DNA accounted for on a dry weight basis is found in the residue, while 90% of the total RNA is retained in the supernatant. The active residue is characterized by Feulgen "positive" (purplish-pink) bodies embedded in cellular material precipitated during hydrolysis with 0.1 *N* HCl (60°, 10 min).

TABLE II

PROTEIN, NUCLEIC ACID (PHOSPHORUS) AND TOTAL PHOSPHORUS CONTENT (mg) OF 12,000 *g* SEDIMENT AND 12,000 *g* SUPERNATANT OF r y + CELLS

200 mg dry weight of PF

Component	*12,000 g Sediment*	*12,000 g Supernatant*
Protein	20.64	40.94
RNA-P	0.932	0.241
DNA-P	0.024	0.00
Total P	0.231	2.34
Activity (r + y + /10⁸)	5.400	0
Feulgen "+" bodies	abundant	rare

For activity studies, one ml of PF is added to 8.0 ml of broth containing 10^8 r+ y recipient cells (stock 101 te-p) genetically incapable of mating with donor cells; appropriate controls are also run (minus PF; minus cells). After recipient cells have undergone *ca.* 3 divisions, the mixture is centrifuged, washed, and plated on lactate nutrient agar which is totally selective against r+ y and r y + cells, permitting only the prototroph to grow. The prototroph frequencies obtained in several experiments are shown in Tables III and IV and are to be compared with a frequency of approximately 1 per 10^7 observed when intact donor and intact recipient cells were grown together. The possible contribution of rare, illegitimate fusions between recipient cells and donor cells in the homogenate or 12,000 *g* residue was tested by appropriate

TABLE III

ULTRAVIOLET INACTIVATION AND ATTEMPTED PHOTOREACTIVATION OF THE ACTIVITY OF THE 12,000 *g* RESIDUE FROM r y + CELLS (STOCK 8256.27). THE PARTICULATE FRACTION WAS SUBJECTED TO THE ULTRAVIOLET DOSES INDICATED (6.0 ergs/mm²/sec) AND THEN MIXED WITH te-p RECIPIENT CELLS AS DESCRIBED IN THE TEXT

	No. of prototrophs		*% Activity*	
	Dark	*+ PR*	*Dark*	*+ PR*
te-p cells only	0/10⁸	0/10⁸	—	—
Particulate fraction (PF) only	0	0	—	—
te-p + PF (0 sec UV)	3940/10⁸	3900/10⁸	100.00	99.98
+ PF (200 sec UV)	1340/10⁸	1650/10⁸	34.10	42.31
+ PF (400 sec UV)	89/10⁸	54/10⁸	2.26	1.38
+ PF (600 sec UV)	17/10⁸	20/10⁸	0.43	0.51
+ PF (1000 sec UV)	1/10⁸	0/10⁸	0.03	0.00
+ PF (1500 sec UV)	0/10⁸	0/10⁸	0.00	0.00

TABLE IV

THE FREQUENCY AND PHENOTYPE OF RESPIRATION-SUFFICIENT (r + y+) PROTOTROPHS ARISING FROM MIXTURES OF INTACT r + y CELLS AND HOMOGENATES OF r y + CELLS

Flask	Strain and phenotype	Frequency of prototrophs			
	Experiment	1	2	3	4
I	8256.27 (r y +) z + ac + ca + cu + pa − th − ad − (homogenate)	$0/10^8$	$0/10^8$	$0/10^8$	$0/10^8$
II	101 te-p (r + y) z − ac − ca − cu − pa + th + ad + (intact cells)	$0/10^8$	$0/10^8$	$0/10^8$	$0/10^8$
III	homogenate of 8256.27 + intact cells of 101 te-p	$54/10^5$	$230/10^5$	$64/10^5$	$78/10^4$

Analysis of prototrophs from flask III (above). All isolates (505) arising from 10^{-5} and 10^{-6} dilutions plated on lactate were tested

Phenotype	Number of isolates
z − ac − ca − cu − pa + th + ad +	505
z + ac + ca + cu + pa − th − ad −	0
other combinations	0

z + (z): ability (inability) to ferment melezitose.
ac + (ac), ca + (ca), cu + (cu): resistance (sensitivity) to 2 actidione/ml, 0.8% caffeine, and 0.17% copper sulfate, respectively.
ad + (ad), th + (th), pa + (pa): ability (inability) to grow on synthetic medium without adenine, thiamine, and pantothenate, respectively.

genetic markers and tetrad analysis: Recipient cells of r + y zac ca cu ad + th + pa + constitution, when incubated with homogenates of r y + z + ac + ca + cu + ad − th − pa − cells, gave rise to r + y + z ac ca cu ad + th + pa + prototrophs only (Table IV). Tetrad analysis of 36 asci of three independent prototrophs failed to show the presence of the z + ac + ca + cu + markers which are expressed as dominant traits in both diploid and triploid hybrids. None of 4000 prototrophs tested carried the 4 dominant markers, showing that illegitimate fusion between donor and recipient cells (although it may occur rarely) cannot explain the transfer of respiratory competence to the r + y stock.

Under appropriate conditions, the prototrophs may be used to prepare active particulate fractions. The activity of the 12,000 *g* residue is markedly reduced by high concentrations of nucleases but only weakly affected by proteolytic enzyme[2]. These and other studies show that the particulate fraction contains a "biologically active" factor which appears to be associated with a nucleoprotein fraction of the cell. The fact that active fractions are obtained with r y+ stocks but not from r y mutants (otherwise isogenic) identifies the activity with the genetically-defined y+ trait.

Table III shows that the activity of the particulate fraction is markedly decreased by ultraviolet radiation when irradiated in $M/15$ KH_2PO_4 (pH 6.0). Within experimental error, there is no PR of activity. Addition of supernatant of the r y+ stock has no effect on the inactivation rate with or without PR. These results are consistent with the general lack of PR of virus and biomolecules in a simple medium. However,

References p. 304

on genetic grounds alone we would expect no PR of activity of the subcellular fractions treated with supernatant of r y+ stocks since they lack the postulated cellular component required for PR of mutation in the intact cell. The obvious question under study is whether Rupert's enzyme[3] or extracts of r+ stocks will effect PR of the particulate fraction in the presence of recipient cells.

Although elucidation of the photoreactivation process described here awaits further studies, I believe the results offer promising attacks on the nature of ultraviolet and photoreactivation processes in general, and specifically on the question of the identity of radiation processes leading to mutation and killing[7]. An optimistic approach is the screening of coliform and yeast cultures for mutants deficient for Rupert's enzyme and for cellular components necessary in the photoreactivation of mutation and killing.

REFERENCES

1 A. Kelner, *J. Bacteriol.*, 65 (1953) 252.
2 D. Pittman, D. Perry, F. Wilson and L. E. Coker, in preparation.
3 C. S. Rupert, S. H. Goodgal and R. M. Herriott, *J. Gen. Physiol.*, 45 (1958) 451; C. S. Rupert, *J. Gen. Physiol.*, 43 (1960) 573.
4 D. Pittman, J. M. Webb, A. Roshanmanesh and L. E. Coker, *Genetics*, 45 (1960) 1023.
5 B. Ephrussi, *Nucleo-Cytoplasmic Relations in Microorganisms*, Oxford University Press, London, 1953.
6 F. Wilson, *Thesis*, Southern Illinois University, 1958.
7 M. R. Zelle, J. E. Ogg and A. Hollaender, *J. Bacteriol.*, 75 (1958) 190.

MACROMOLECULAR SYNTHESIS IN A THYMINE-REQUIRING BACTERIUM AFTER ULTRAVIOLET IRRADIATION

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Quantitative studies have been made on the effects of ultraviolet light (U.V.) on colony formation, protein synthesis, RNA synthesis, and DNA synthesis in *Escherichia coli* 15T-. The initial studies (done at Yale University, Biophysics Dept.) were performed on exponentially-growing cultures, suspended in "tris" buffer for the irradiations at 37° and resuspended in growth medium afterwards[1]. A water-prism monochromator was used for U.V. source (2652 Å) and intensities were measured by calibrated phototube. The reported doses below represent average doses received, as calculated for the known bacterial concentration.

The dose-survival curve for colony forming ability (platings by spreading on nutrient agar and immediate incubation at 37°) was a simple exponential function, yielding 37% survival after a dose of 65 ergs/mm^2.

Protein synthesis was followed by the incorporation of ^{35}S (from $^{35}SO_4$) into the trichloroacetic acid-insoluble fraction of the bacteria. Linear incorporation of ^{35}S was obtained for at least the first hour of growth after U.V. doses above 150 ergs/mm^2. Higher doses gave linear incorporation curves with lower slopes A dose-effect curve, constructed by comparing the slopes of these linear incorporations. yielded a simple exponential survival for protein synthesis, with 37% survival after 160 ergs/mm^2.

RNA and DNA synthesis were followed by the incorporation of ^{32}P (from $^{32}PO_4$) into the respective fractions[2]. Incorporation of ^{32}P into RNA was also linear after U.V. doses above about 150 ergs/mm^2, but the effect on RNA synthesis could not be represented by a "one hit" dose-effect curve. (For doses below 60 ergs/mm^2 the ^{32}P incorporation was always within experimental error of that for an unirradiated culture.) The final exponential slopes of the dose-effect curves were the same for RNA as for protein synthesis, but the RNA curve extrapolated back to give a multiplicity of 1.5 . 37% survival of RNA synthesis occurred after 240 ergs/mm^2. DNA synthesis exhibited a temporary delay after irradiation, before resuming at the control rate. The delay was roughly proportional to the dose and a dose of 150 ergs/mm^2 inhibited DNA synthesis for abour one division period (50 min). No difference in the effect on either protein or RNA synthesis was found when thymine was withheld from the growth medium after irradiation[1]. Both protein and RNA synthesis were photoreactivated by subsequent exposure of the irradiated culture to high intensity visible light, whether or not thymine was present[3].

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References p. 307

More recently, Prof. Ole Maaløe and I have been studying some properties of the triple mutant, *E. coli* 15T-A-U-, requiring thymine, arginine, and uracil[4]. We have found that when all three requirements are removed, thymineless death proceeds only to the 3% survival level. After preincubation for 90 min with thymine (-A-U) the entire population is immune to thymineless death (-T-A-U). This finding and subsequent experiments led to our suggestion that the bacterial DNA replication cycle could run to completion in the absence of protein synthesis, but that a new round of replication could not be initiated until conditions permitting protein synthesis were reestablished[4].

A number of reported studies on irradiated bacteria have shown that sensitivity to U.V. decreases markedly after conditions in which protein synthesis is inhibited while DNA synthesis continues. Billen has shown that sensitivity of *E. coli* 15T- to X-rays is decreased after incubation with chloramphenicol, and he has postulated that the "excess" DNA formed may be responsible for the effect[5]. Our results with the triple mutant would suggest an alternative possibility, that the decrease in sensitivity is due to a change in *state* of the DNA.

To check this hypothesis we have performed a number of U.V. experiments with triple mutant in different growth states. These irradiations were performed with a germicidal U.V. lamp and the incident intensities were measured with a Laterjet dosimeter. Irradiations were carried out on cultures suspended in "tris" buffer at room temperature.

Protein synthesis (^{35}S incorporation, as before) exhibited the same sensitivity to U.V. in a exponentially growing culture of *E. coli* 15T-A-U- as in a culture which had received the 90-min preincubation +T-A-U prior to irradiation. It should be mentioned that the control incorporation rates were the same in both cultures (*i.e.* after the 90-min inhibition, protein synthesis can begin immediately on addition of the required arginine and uracil). Thus, the changes which occur during the preincubation period apparently do not affect *quantitatively* the ability of irradiated cells to synthesize protein after a given U.V. dose.

Colony formation showed a very striking difference in sensitivity to U.V. after the preincubation +T-A-U. An exponentially-growing culture exhibited the same characteristics as the parent strain described earlier. However, after 45 min preincubation +T-A-U (by which time 35% of the population is immune to thymineless death) a multiple-target type dose-effect curve was obtained, with the same final exponential decay rate, but a multiplicity of 10. After the full 90-min preincubation, the multiplicity had increased to the order of 100. The increment in DNA of about 40% during the preincubation period is not sufficient to account for such a striking decrease in sensitivity and it seems more likely that the effect is due to the existence of a less labile state of the DNA at some stage in its replication cycle. Also, it would appear that the transition to the resistant state is not a gradual one occurring throughout the growth cycle, or one would not expect to find the simple exponential survival curve for an exponentially growing culture.

Since the U.V. absorption cross-section for nucleic acid is about 20 times that for protein and since there is about 6 times as much RNA as DNA in the *E. coli* cell, the U.V. absorption is predominantly due to the ribosomes which contain most of the RNA. It is also expected that at some stage in the growth cycle the RNA may be associated with the DNA to obtain its specificity. Suppose that this association and

transfer of information occurs only during the active synthesis period of the DNA replication cycle and that the RNA units become dissociated at the completion of the cycle. The U.V. damage to DNA may be due primarily to photons absorbed in associated RNA units. When the RNA is associated with the DNA a "hit" in any unit is likely to inactivate some essential function and lead to inability to form a colony. When the RNA units are dissociated there may be much duplication (*i.e.* many units with the same information) of the different functions, so a large number of ribosomes of a particular type must be inactivated in order to completely inactivate a particular essential function. It is suggested that the sensitivity for survival of bacterial colony formation is determined by the degree of association which exists between the RNA and DNA at the time of irradiation.

ACKNOWLEDGEMENTS

I wish to express my gratitude to Prof. R. B. Setlow for helpful discussion and encouragement during the early phases of this work. I should also like to express my appreciation to Prof. O. Maaløe for the very stimulating period of study and collaboration in his laboratory in Copenhagen during my tenure on a post-doctoral fellowship from the U.S. Public Health Service.

REFERENCES

1 P. C. Hanawalt and R. B. Setlow, *Biochim. Biophys. Acta*, 41 (1960) 283.
2 P. C. Hanawalt, *Science*, 130 (1959) 386.
3 P. C. Hanawalt and J. Buehler, *Biochim. Biophys. Acta*, 37 (1960) 141.
4 O. Maaløe and P. C. Hanawalt, *J. Molec. Biol.*, (in the press).
5 D. Billen, *Biochim. Biophys. Acta*, 34 (1959) 110.

ULTRAVIOLET EFFECTS AND PHOTOREACTIVATION IN ISOLATED CELL FRACTIONS*

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These experiments were designed to test whether or not any mechanism similar to that described by Rupert[1] for the photoreactivation (PR) of the transforming activity of deoxyribonucleic acid (DNA) could be demonstrated for the repair of ultraviolet (U.V.) inactivated enzymes and whether or not isolated cell particulates could be photoreactivated. DNA polymerase and the isolated nucleus were selected for study because the nucleus and DNA are considered as probable sites of U.V. damage in the cell. Also, the relative U.V. sensitivity of DNA synthesis by the nucleus and the polymerase could be studied.

The thymus nucleus has the additional advantage that protein synthesis is reduced when the DNA is removed and restored by the addition of DNA (Allfrey *et al.*[2]). Therefore, the U.V. sensitivity of the nucleus can be studied in the absence of DNA with the subsequent addition of irradiated or non-irradiated nucleic acid.

EXPERIMENTAL

DNA polymerase activity was studied in the high speed supernatant of calf thymus homogenate following the Assay Method III described by Bollum and Potter[3]. The supernatant contained 4.7 mg/ml protein. Heat-denatured thymus DNA (Worthington Biochemicals) was used as a primer (Bollum[4]).

Isolation of calf thymus nuclei and $1\text{-}^{14}C$ alanine incorporation studies followed essentially the method of Allfrey *et al.*[2]. All studies were done at a pH of 7.1. For ^{3}H thymidine incorporation studies with the nuclei, the ^{14}C alanine was replaced by the mononucleotide mixture employed by Bollum and Potter[3] with the DNA polymerase.

Samples were counted with a Nuclear-Chicago D-47 counter operated windowless for ^{3}H and with a micromil window for ^{14}C. DNA determinations were done by Burton's[5] modification of the diphenylamine reaction using Worthington Biochemicals thymus DNA as a standard. Protein was determined with a Biuret reagent (Gornall *et al.*[6]).

For U.V. exposures, the samples were placed between two quartz plates (15 cm $\times$ 15 cm $\times$ 0.15 cm) and irradiated from both sides with two 15-W G. E. Germicidal lamps. The sample thickness was 0.16 mm for the enzyme preparation and 0.32 mm for the nuclear suspension. The samples transmitted 20–30% of the incident U.V.

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Exposures were measured with a photocell of the sort described by Jagger[7]. General Electric Company 15-W BLB black lights and a 550-W projector were used as sources for photoreactivating light.

RESULTS

U.V. inactivation curves for the incorporation of 1-^{14}C-alanine and ^{3}H-thymidine by thymus nuclei and of ^{3}H-thymidine by soluble DNA polymerase are shown in Fig. 1 and Fig. 2. Inactivation is exponential, provided sufficiently thin samples are used. The nucleus shows a similar sensitivity to U.V. for both amino acid and thymidine incorporation. The nucleus is 70 times as sensitive to U.V. as the polymerase.

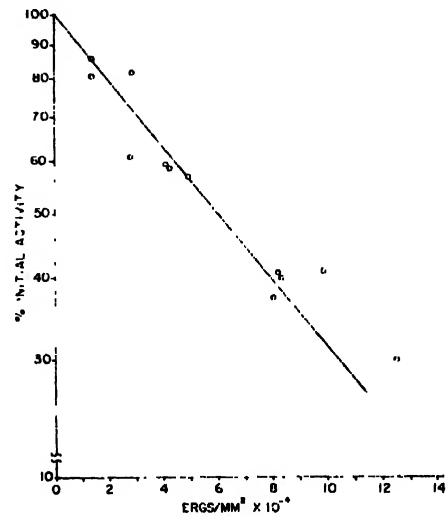

Fig. 1. The effect of U.V. light on ^{3}H-thymidine incorporation by DNA polymerase. Each tube had a final volume of 1 ml and contained 0.3 ml supernatant fraction (1.4 mg protein), 1 mg DNA, 40 mM Tris: HCl pH 8 and 0.3 ml of an enzymatically prepared mixture of deoxyribotide triphosphates (Bollum and Potter[3]) Incubation time 120 min at 38°.

Typical values for incorporation by unirradiated sample were: 300 cts/min/mg protein for ^{14}C by the nucleus, $5 \cdot 10^{3}$ cts/min/mg DNA for ^{3}H by the nucleus, and $4 \cdot 10^{5}$ cts/min/mg DNA for ^{3}H by the polymerase. Under the conditions employed in the experiments, incorporation was very nearly proportional to enzyme or nuclear concentration and to incubation time.

The soluble DNA polymerase system did not photoreactivate under the conditions of the experiments (up to 10^{6} ergs/mm^{2} with BLB bulbs after U.V. inactivation). Alanine incorporation by U.V. irradiated nuclei was increased about 30% by exposure to photoreactivating light. Addition of 1 mg of DNA primer to nuclei increased alanine incorporation by 30-40% in unirradiated and U.V. inactivated nuclei.

DISCUSSION

A comparison of the sensitivity to U.V. of the soluble polymerase and nuclei suggests that damage to the enzyme is probably not a factor in U.V. inhibition of DNA synthesis *in vivo*. This follows from the very large exposures required to affect incorporation in the soluble system. The nucleus, by contrast, is quite sensitive. The block in synthesis must, therefore, be at a different level than the polymerizing enzyme.

Since both protein and DNA synthesis in the nucleus show a similar sensitivity to

References p. 310

U.V., it appears that a common site may be involved. DNA is required for protein synthesis by the isolated nucleus (Alfrey *et al.*[2]) and as primer for the DNA polymerase (Bollum and Potter[3]). There is no evidence, from our work, that the U.V. injury is to the DNA. Addition of DNA to the irradiated nuclei did stimulate ^{14}C uptake; however, a comparable stimulation was observed in unirradiated nuclei.

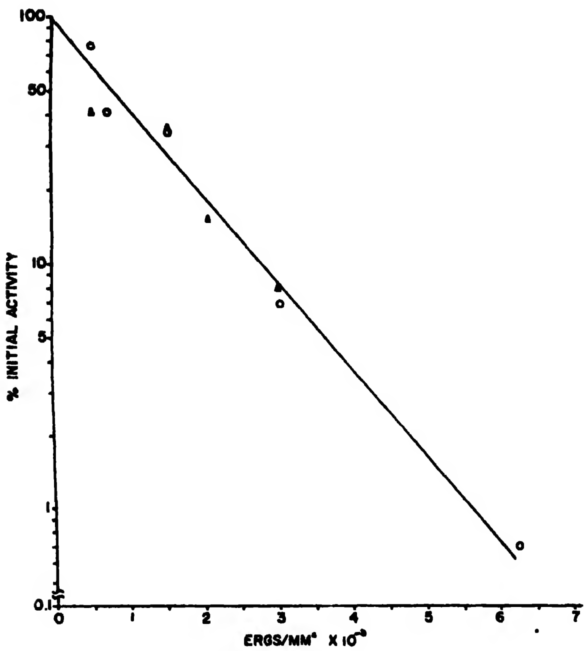

Fig. 2. The effect of U.V. light on 1-^{14}C alanine (o) and ^{3}H thymidine (▲) incorporation by thymus nuclei. Each tube had a final volume of 2 ml and contained 1.0 ml nuclear suspension (5 mg protein dry weight). 0.4 ml of 0.1 *M* sodium phosphate, 0.25 *M* sucrose buffer pH 7.1, 0.4 ml of 0.1 *M* glucose solution containing 3.75 mg NaCl/ml and 0.1 ml H_2O containing either 0.056 m*M* DL-alanine-1-^{14}C (specific activity 0.25 mc/m*M*) or 0.0064 m*M* ^{3}H-thymidine (specific activity 0.39 c/m*M*) and mixture of deoxyribonucleotides. Incubation time 90 min at 38°.

The degree of PR in the nuclei was small, so that work with the system would be difficult. Logan *et al.*[8] observed reversal of U.V. inhibition of phenyalanine incorporation by mixtures of rat liver nuclei and mitochondria. It is not clear whether the processes are similar since they used a two component system and obtained the same effect by irradiating either of the components.

ACKNOWLEDGEMENT

The authors wish to thank Dr. John Jagger for his advice and for help in the calibration of sources.

REFERENCES

1 Claude S. Rupert, *J. Gen. Physiol.*, 43 (1960) 573.
2 V. G. Allfrey, A. E. Mirsky and Syozo Osawa, *J. Gen. Physiol.*, 40 (1957) 451.
3 F. J. Bollum and Van R. Potter, *J. Biol. Chem.*, 233 (1958) 478.
4 F. J. Bollum, *J. Biol. Chem.*, 234 (1959) 2733.
5 K. Burton, *Biochem. J.*, 62 (1956) 315.
6 A. G. Gornall, C. J. Bardawill and M. M. David, *J. Biol. Chem.*, 177 (1949) 751.
7 J. Jagger, in Press (1960).
8 R. Logan, M. Errera and A. Ficq, *Biochim. Biophys. Acta*, 32 (1959) 147.

NOTE ADDED IN PROOF

More thorough inactivation studies with nuclei have now been done. The results show that ^{3}H-thymidine incorporation is somewhat more sensitive to U.V. than ^{14}C-amino acid incorporation.

PHOTOENZYMATIC REPAIR OF ULTRAVIOLET DAMAGE IN DNA

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SUMMARY OF LECTURE

Ultraviolet damaged bacterial transforming DNA can be photoreactivated *in vitro* by enzyme systems obtained from photoreactivable cells. The main details of this phenomenon have been reported previously[1,2]. It supports the hypothesis that photoreactivation of cells involves repair of ultraviolet damage to cellular DNA.

Early experiments with the *E. coli* enzyme system suggested the existence of two components — one dialyzable and heat stable which was used up in the reaction, and one non-dialyzable and heat labile which was not consumed. Extending these experiments to a wider range of reaction conditions has shown that no stoichiometric relation exists between the amount of this supposed dialyzable component and the amount of DNA repaired[3], although preparations lose activity upon dialysis and recover upon adding back concentrated dialysate[1,4]. Attempts at purification of this *E. coli* system have been frustrated by the appearance of nuclease activity in all fractions. The photoreactivating enzyme system from baker's yeast, which is readily purified and with which most of the work has been done, shows no evidence of any component used up in the reaction. It apparently consists of a single enzyme[2].

The yeast enzyme in the dark forms a stable complex with U.V. irradiated DNA[3]. This sediments in an ultracentrifugal field at the high rate characteristic of DNA rather than the slower rate characteristic of the free enzyme. The complex dissociates in the light to generate a repaired DNA structure and free enzyme, according to the conventional scheme

$$E + S \underset{k_2}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_3(I)} E + P$$

where E represents the enzyme, S the substrate (U.V. damage in DNA), ES the complex and P the product (a repaired DNA structure). $k_3(I)$, dependent on the light intensity, is zero in the dark. At constant light intensity the reaction follows (at least approximately) Michaelis–Menten kinetics.

This photoenzyme system bears a striking formal analogy to the retinene–opsin–rhodopsin system of vertebrate rod vision, although the two systems are chemically quite different[3]. The same formal pattern may hold for the primary events underlying other photobiological processes.

The photoreactivating enzyme permits the longer wavelengths of sunlight to bring about a repair reaction counteracting the damage done to DNA by the shorter wave-

References p. 312

lengths Transforming DNA inactivated by solar radiation in a quartz flask partially recovers in sunlight in the same quartz flask when a little photoreactivating enzyme is added[3]. This may represent the original utility of photoreactivation to an organism.

The presence of photoreactivable damage in U.V. irradiated non-transforming DNA may be detected by its competitive inhibition of transforming DNA repair. This inhibitory power is eliminated by allowing the enzyme to act on the irradiated competing DNA first[2,3]. By these criteria, photoreactivable damage develops in all kinds of mammalian, bacterial and viral DNA tested, including single-stranded ϕX-174 DNA. It also develops in heat, acid and alkali denatured DNA, and once present, survives in DNA treated at 100° for 40 min or at pH 2.5 or 12.5 overnight at room temperature. It does not develop when the actual irradiation of denatured DNA occurs at a pH below 3.0-3.5, the region in which purine and pyrimidine amino groups become protonated. No competitive inhibition has been found with irradiated RNA.

REFERENCES

1 C. S. Rupert, S. H. Goodgal and R. M. Herriott, *J. Gen. Physiol.*, 41 (1958) 451.
2 C. S. Rupert, *J. Gen. Physiol.*, 43 (1960) 573.
3 C. S. Ruppert, in M. Burton, J. S. Kirby-Smith and J. L. Magee (editors), *Comparative Effects of Radiation*, John Wiley, New York, 1960, p. 49.

PHOTOREACTIVATION OF THE ULTRAVIOLET DAMAGED STREPTOMYCIN RESISTANCE MARKER IN DNA OF STREPTOCOCCI AND PNEUMOCOCCI

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When we submitted the preliminary communication to this Congress we had been unable to demonstrate photoreactivation of U.V.-irradiated streptococcal and pneumococcal DNA. Since then however, we have succeeded in photoreactivating both these transforming activities.

At the outset we used *E. coli* extracts prepared from bacteria grown for 24-26 h followed by grinding with quartz sand until over 90% of cells had been disrupted. With different batches of these extracts, designated No. 1, a photoreactivation effect of 15-55% could easily be produced in U.V. irradiated DNA of *Hemophilus influenzae*. However all these extracts were non-active in photoreactivation of streptococcal and pneumococcal DNA and in addition they exerted a strong inhibiting effect on transformation of the cocci. The number of transformants for both intact and irradiated DNA was reduced by more than 90%. This inhibition was found to be due mainly to the abundant amounts of *E. coli* DNA present in the extracts. This DNA competes for competent cells with transforming DNA of streptococci and pneumococci but not of *H. influenzae*. Various attempts to remove the inhibitory DNA from the extracts without destroying the factor necessary for photoreactivation were unsuccessful.

TABLE I

ACTIVITY OF *E. coli* EXTRACTS NO. 2 IN PHOTOREACTIVATION OF U.V. DAMAGED STREPTOCOCCAL AND PNEUMOCOCCAL DNA

DNA used in transformation	*Number of transformants*							
	Streptococcus E. coli extracts				*Pneumococcus E. coli extracts*			
	31		*32*		*31*		*32*	
Non-irradiated	$142 \cdot 10^4$	%	$176 \cdot 10^4$	%	$89 \cdot 10^3$	%	$89 \cdot 10^3$	%
U.V.-irradiated	$184 \cdot 10^3$	100 %	$107 \cdot 10^3$	100 %	$453 \cdot 10^1$	100 %	$453 \cdot 10^1$	100 %
With *E. coli* extract, dark	$50 \cdot 10^2$	2.7 %	$48 \cdot 10^2$	4.5 %	$118 \cdot 10^1$	26 %	$231 \cdot 10^1$	51 %
With *E. coli* extract, illuminated	$105 \cdot 10^2$	5.7 %	$90 \cdot 10^2$	8.3 %	$541 \cdot 10^1$	119 %	$601 \cdot 10^1$	133 %
Photoreactivation increase	2.1 times		1.8 times		4.6 times		2.6 times	

The negative results stimulated a change in the method of preparing the *E. coli* extracts. Bacteria were grown for only 8–12 hours, ground to destroy 30–40% of cells and extracted with distilled water or phosphate buffer. This type of extract, designated No. 2 was less inhibiting in transformation of pneumococci but did not differ significantly in this respect with respect to transformation of streptococci. With many of these extracts a slight but quite distinct photoreactivation effect could be produced (Table I).

It will be seen that the activity of photoreactivated streptococcal DNA is below that of the U.V.-irradiated DNA. This is certainly due to the inhibiting effect of the *E. coli* extracts. We think however that the differences observed between the activities of U.V.-irradiated DNA and *E. coli* extract kept in the dark and the same mixtures illuminated with visible light are really due to photoreactivation. The basis for this is that in no case did visible light illumination of mixtures of U.V. non-irradiated DNA and *E. coli* extract cause any increase of transforming activity as compared with activity of the same mixtures kept in the dark.

No explanation was found for the fact that both kinds of extract, No. 1 and No. 2, are active in photoreactivation of U.V.-irradiated DNA of *H. influenzae* and only the latter one in photoreactivation of coccal DNA's.

PHOTOREACTIVATION AND REVERSIBLE PHOTOLYSIS OF NUCLEIC ACIDS

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The proposal that biological photoreactivation (PR) is due to a reversal of ultraviolet (U.V.) damage to essential nucleic acid molecules is by no means new. Its essence may be found in an early review of the subject by Dulbecco[1] and it has been more explicitly formulated in the recent review of Jagger[2] as the most probable of the various theories extant in the field. What is new is the accumulation during the past few years of a considerable mass of new experimental data on the photochemistry of nucleic acids and their derivatives, as well as on model oligo- and polynucleotides, which has laid the groundwork for an interpretation on a macromolecular scale of the origin of photobiological effects in general and of PR in particular.

The experimental facts relating to biological PR have been extensively reviewed by Jagger[2] and will undoubtedly be covered in Dr. Kelner's talk at this symposium; hence only a few salient features will be mentioned here:

(*a*) PR embraces reproduction, mutation and transformation, all of which involve DNA-protein. There are also several authentic examples of cytoplasmic PR which are hence likely to involve RNA-protein. Action spectra indicate that the primary receptors are NA[3], with some evidence for participation of proteins. However, the well-known ability of U.V. to destroy virus infectivity without affecting antigenic properties suggests that the effect is largely on NA.

(*b*) For bacteriophage there is no doubt about DNA-protein being involved and the evidence is in favour of DNA. Earlier doubts about the authenticity of PR for plant viruses, *i.e.* RNA-protein, have been dispelled by Bawden and Kleczkowski's[4] demonstration of PR for RNA of TMV. Taken together with Rupert, Goodgal and Herriot's[5] discovery of extracellular PR for transforming DNA (T-DNA), this establishes beyond doubt that each type of NA alone exhibits PR.

(*c*) The available evidence indicates that PR operates predominantly via reversal of U.V. damage rather than by the initiation of alternative metabolic pathways.

We may therefore pose the following question: What are the chemical and/or physico-chemical modifications induced in nucleic acids by doses of U.V. not markedly in excess of those resulting in biological effects; and are such modifications reversible, in whole or in part?

The answer to this is that we now have a fair idea as to what happens to irradiated NA and that the resulting effects, for biologically effective doses of radiation (of the order of 10^{16}–10^{17} quanta/cm^2/min or 10–100 ergs/mm^2/sec), exhibit physico-chemical reversibility to the same degree as biological PR[6–9].

References p. 319

Physico-chemical studies of nucleic acids and viruses subjected to biological doses of radiation show that:

(*a*) There is no rupture of internucleotide linkages.

(*b*) There is no change in viscosity or intrinsic viscosity.

(*c*) There is no change in molecular weight.

(*d*) There is only a detectable drop in U.V. absorption.

(*e*) For T-DNA there is a slight drop in the temperature of the transition profile[10], suggesting rupture of a very few hydrogen bonds in the twin-stranded structure.

From the above facts it may be concluded that the resulting damage to irradiated NA molecules must be highly localized in character.

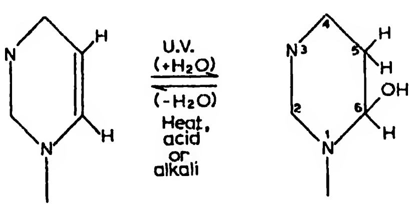
Chemical effects of irradiation are as follows:

(*a*) Carbohydrate moieties are unaffected.

(*b*) Spectroscopic and chemical evidence indicates the lack of any effect on the purine bases (additional confirmation of this would be desirable).

(*c*) There is, in addition, some evidence from action spectra that in single-stranded DNA only the pyrimidine components are affected[11].

(*d*) Free uridylic (Up) and cytidylic (Cp) acids are transformed by U.V. to the 5-hydro-6-hydroxy derivatives and these photoproducts may revert to the parent substances in almost 100% yield as follows:



(*e*) Free thymidylic acid (Tp) undergoes degradation. We shall refer to 5-methylcytidylic acid and its analogues separately below.

(*f*) In single stranded polynucleotide chains, Tp residues apparently undergo irreversible photolysis. Up and Cp residues flanked by purines are transformed as in (*d*), above, and subsequently exhibit 70–100% reversibility. In tracts of Up residues reversibility may vary from 80–65% and in tracts of Cp residues is about 90%. The remaining Up and Cp residues in such tracts apparently give rise to non-reversible photoproducts, probably due to cross-linking between the 5–5 and 6–6 positions of adjacent pyrimidine rings. This is the predominant reaction in irradiated dry films of NA[12] and explains why it is not possible to photoreactivate irradiated dried phage films. It is also unlikely that irradiated dried films of T-DNA or infectious RNA would exhibit PR.

(*g*) In twin stranded model polynucleotide chains the quantum yield for Up residues is only ¼ of that in single stranded chains, but the % reversibility is the same. Both the quantum yield and % reversibility for Cp residues are unaltered in going from a single to a twin stranded chain.

(*h*) In RNA from natural sources, spectral evidence shows that 50% of the damage inflicted by U.V. may be subsequently reversed by heat.

Summing up, it may be seen that one can obtain physico-chemical reversal of the

effects of U.V. on natural and model polynucleotide chains to the extent of 50% or more of the damage inflicted, or about that prevailing for biological PR. Reversible photolysis of pyrimidine residues in nucleic acids consequently provides a reasonable experimental analogue for biological PR. The principle criticism which may be levelled at this model is that reversibility has hitherto been obtained mainly by heating at neutral pH, *i.e.* it is thermal reversibility (TR) and not PR.

It is on the other hand well-known that agents other than visible light may be used to reverse the effects of U.V. on biological systems and that, of all these, the one that most closely resembles PR is TR. Each of these may therefore be considered as a different source of energy for effecting the reverse reaction. It is of some significance in this regard that the light requirements in bacterial photosynthesis may be completely replaced by ATP in the dark[13]. Furthermore biological PR is accompanied by dark reactions with activation energies of about 9–18 kcal/mole as compared to physico-chemical TR for which the values are 14–26 kcal/mole.

It is perhaps pertinent to the above argument that uridine, following U.V. destruction of its properties as a growth factor, may regain 100% of its biological activity by acidification, alkalization, or heating (TR) at neutral pH. The photoproduct of the pyrimidine component of vitamin B_1 (2-methyl-5-ethoxymethyl-4 aminopyrimidine) may also regain its full biological activity by TR.

Particularly striking is the little-known observation of Bresch[14] that irradiated phage T1 adsorbed to *E. coli* exhibits a small, but definite, TR. Miss Lukjaniec has confirmed this in our laboratories and demonstrated TR for several other phages as well. No TR could be obtained for non-adsorbed phage at elevated temperatures because of denaturation, but this might be well worth attempting with T-DNA which has a much higher denaturation temperature.

The following may therefore be regarded as a rather rough picture of what happens to an irradiated nucleic acid chain:

In both single and twin stranded chains in those regions which contain tracts of pyrimidines, a fraction of the latter undergo a reaction which is non-reversible by acid or heat and is due to cross-linking of the 5,6 double bonds of adjacent pairs of pyrimidines. The majority of them as well as many of those distributed randomly in the chain become hydrated at the 5,6 double bond. In a single stranded chain the secondary structure may be slightly modified as a result of hydrogen bond breakage to an affected base. In a twin stranded chain some of the interchain hydrogen bonds would also be ruptured (we have found, *e.g.* that irradiated poly-U will not form a twin stranded complex with poly-A). The net result is a localized modification of base sequences accompanied by a slight loss in secondary structure for a single stranded chain and in secondary structure and interchain bonding for a twin strand.

How many bases are affected and how many hydrogen bonds broken? From what has been pointed out above, the number must be very few. It can be estimated very approximately in two ways from existing data: (*a*) from the decrease in U.V. absorption of inactivated RNA from TMV[15], and from quantum yield data for the same RNA[15] as well as for the genetic markers of pneumococcal T-DNA[16], it would appear that inactivation is accompanied by the photochemical transformation of about 1 residue in 200–400; (*b*) from the observations of Marmur and Doty[10] on the dependence of DNA denaturation temperature on the % guanine-cytosine base pairs, and the finding of the same authors that inactivation of pneumococcal T-DNA is accom-

References p. 319

panied by a 1.5° decrease in this temperature, it may be estimated that 1, or at most 2, base residues per 200 are affected for complete inactivation. These figures are to be compared with the observations of Freese[17] on reverting point mutations in phage T_4 induced by chemical agents or base analogues, from which it was concluded that each mutation is due to the modification of a small number, and probably only one, base pair.

The dependence of the quantum yield, as well as the activation energy and rate of TR, for a given pyrimidine residue on the nature of the bases flanking it also makes it possible to explain qualitatively the differences in sensitivities of different markers and of different organisms, the complex nature of some inactivation curves and the variation in activation energies for TR and PR. For example the kinetics of TR for *E. coli*, which have been treated by Buzzell[18] in terms of the "poison theory", may be quite reasonably interpreted in terms of TR of pyrimidine residues[19]. Extension of photochemical studies to a wider variety of base sequences in model polynucleotides should considerably improve the ability to interpret sensitivities and inactivation kinetics (see final paragraph below).

In terms of the above scheme the PR enzyme system of Rupert *et al.*[5] would be expected to act on irradiated DNA by removing a water molecule from the 5,6 bond of certain pyrimidine residues (see also final paragraph). Attempts have been made in our laboratory by Miss C. Janion to demonstrate such a reaction using nucleotide photoproducts, with negative results, perhaps because the presence of internucleotide linkages is a prerequisite for the specificity of the enzyme. If it should prove feasible to purify the PR enzyme, one might expect to learn something about its mode of action by examining its activity against model oligo- and polynucleotides which have been irradiated under conditions such that known modifications have been produced in it.

The *T-even bacteriophages* must be considered separately in relation to the foregoing scheme inasmuch as their pyrimidine constituents include only 5-substituted derivatives, *viz.* thymine, 5-hydroxymethylcytosine and mono- and di-glucosylated 5-hydroxymethylcytosine, which would not normally be expected to give reversible photoproducts. And yet the T-even phages readily exhibit PR. A separate investigation has now shown that all 5-substituted cytosine nucleotides are relatively radiation resistant and that they form photoproducts (as yet unidentified)[20] which, although not susceptible to TR, are relatively radiation resistant and heat stable and contain the *intact pyrimidine ring* so that they are at least potentially reversible by some mechanism. It should therefore be recalled that the pyrimidine component of vitamine B_1 (see above), which is a 5-substituted cytosine analogue exhibits 100% TR following total photochemical transformation. Furthermore, in the work on phage TR referred to above it has been shown that phage T2 exhibits considerably greater TR than phage 1, although it must be admitted that no TR could be found for phage T4. There has been no published report as yet of an extracellular PR enzyme system for bacteriophages; if and when such an enzyme is isolated it would be of interest to examine its behaviour toward the different T-even phages as well as some photoproducts of 5-substituted cytosine nucleotides.

REFERENCES

1 P. Dulbecco, *Radiation Biology*, (ed. A. Hollaender), Vol. II, McGraw-Hill, New York, 1955.
2 J. Jagger, *Bacteriol. Rev.*, 22 (1958) 99.
3 R. Setlow, *Adv. in Biol. and Med. Phys.*, 5 (1957) 37.
4 F. C. Bawden and A. Kleczkowski, *Nature*, 183 (1959) 503.
5 C. S. Rupert, S. H. Goodgal and M. Herriott, *J. Gen. Physiol.*, 41 (1958) 451.
6 D. Shugar and K. L. Wierzchowski, *Postepy Biochem.*, 4, suppl. (1958) 243.
7 K. L. Wierzchowski and D. Shugar, *Acta Biochim. Polon.*, 6 (1959) 313.
8 K. L. Wierzchowski and D. Shugar, *Acta Biochim. Polon.*, 7 (1960) 377.
9 D. Shugar and K. L. Wierzchowski, *Communication at this Congress.*
10 J. Marmur and P. Doty, *Nature*, 183 (1959) 1427.
11 R. Setlow, *Biochim. Biophys. Acta*, 39 (1960) 180.
12 D. Shugar and J. Baranoswka, *Nature*, 185 (1960) 33; *Acta Biochim. Polon.*, 7 (1960) 505.
13 M. Losada, A. V. Trebst, S. Ogata and D. I. Arnon, *Nature*, 186 (1960) 753.
14 V. C. Bresch, *Z. Naturforsch.*, 5b (1950) 420.
15 A. D. McLaren and W. N. Takahashi, *Radiation Research*, 6 (1957) 532.
16 L. S. Lerman and L. J. Tolmach, *Biochim. Biophys. Acta*, 33 (1959) 371.
17 E. Freese, *Brookhaven Symposia in Biol.*, 12 (1959) 63.
18 A. Buzzell, *Arch. Biochem. Biophys.*, 62 (1956) 97.
19 K. L. Wierzchowski, *Doctorate thesis*, 1960.
20 K. L. Wierzchowski and D. Shugar, *Acta Biochim. Polon.*, 7 (1960) 63; and *Communication at this Congress.*

NOTE ADDED IN PROOF

Since submission of the foregoing, concrete evidence has been forthcoming (R. Beukers and W. Berends, *Biochim. Biophys. Acta*, 41 (1960) 550) for the photochemical formation of cross-linkages between 5,6 double bonds of pairs of pyrimidine rings The resulting dimer photoproducts are dissociated by ultraviolet radiation to the original monomers. The possibility must therefore be envisaged that such cross-linking in polynucleotide chains, although not reversible by acid or heat, may be reversed by some other means.

SOME UNSOLVED PROBLEMS ON PHOTOREACTIVATION CONCLUDING REMARKS

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While the progress presented upon many fronts in this symposium indicates resolution of some of the problems of photoreactivation and is a heartening sign of continued interest in the effects of U.V. upon cells, there remain a number of unsolved problems, some of which have been mentioned in the course of the discussions. Attention here is focused upon two; first, why some cytoplasmic effects are subject to photoreversal while others are not; second, why U.V. damage to some species of organisms is not subject to photoreversal.

It appears that effects of U.V. upon the nucleus of a given organism are subject to photoreactivation while effects of U.V. upon cytoplasm may not be. Thus hatchability of eggs of *Habrobracon* is reduced whether the nuclear or cytoplasmic region is irradiated with U.V., but only the nuclear effect is photoreversed[1,2]. Immobilization of *Paramecium* by short U.V. is not subject to photoreactivation, whereas the retarding effect of the same radiations upon division, which is probably chiefly due to an effect of the radiations upon the nucleus of the same animals, is photoreversed[3]. No one has been able to photoreverse the effect of U.V. upon the cell membrane.

On the other hand, some effects which are localized in the cytoplasm are photoreversed. It has been reported that the action of U.V. on the spike potential of nerve fibers which are detached from the nucleated part of the cell[4] is photoreversed. In this symposium Dr. Tageeva has presented data on plant cells in which several effects of U.V. localized in the cytoplasm are photoreversed, the most notable being protoplasmic streaming. Dr. Schiff in this symposium, also reported photoreversal of a cytoplasmic action of U.V., namely inhibition of chloroplast development.

The discrepancy between photoreversibility and non-photoreversibility of various cytoplasmic effects may be resolvable on the basis of action of U.V. at many loci in the cell, action on different loci being responsible for different effects. Action spectra for the U.V. effects upon the cytoplasm of the cell may give pertinent information.

The action spectrum for inhibition of chloroplast development in *Euglena* implicates nucleoproteins. Consequently in this case, the effect of U.V. on cytoplasm may be mediated by reactions much like those following irradiation of the nuclei, and like those in the nuclei, photoreversible. One might postulate similar action spectra and mechanisms for other photoreversible effects of U.V. upon the cytoplasm. The action spectrum for effects of U.V. upon nerve, however, resembles protein absorption, with the alternate suggestion of thiamin as the substance damaged by the U.V.[5].

Action spectra have not yet been determined for the retarding effect of U.V. on protoplasmic streaming and other activities of plant cells reported by Dr. Tageeva.

The action spectrum for immobilization of cilia resembles the absorption spectrum by an unconjugated protein such as globulin[3], suggesting the possibility that the damaging effects of U.V. mediated by cell proteins are not subject to photoreversal. However, the action spectrum for the effect of U.V. on hatchability of *Habrobracon* eggs resembles nucleoprotein absorption regardless of whether the cytoplasm or the nucleus of the eggs is irradiated[1,2]. The confusing problem of the mechanism of cytoplasmic photoreversal therefore awaits further work for its resolution.

The fact that some U.V.-treated cells have shown no photoreactivation has been of concern for some time. Quite a number of bacteria, among them some bacilli (ref.[6], p. 106, 11) *Streptococcus faecalis* and *S. lactis*[7], *Azotobacter vinelandii*[8], *Hemophilus influenzae* and *Diplococcus pneumoniae*[9] show no photoreactivation from U.V. inhibition of growth. Among animal cells, retardation of division of *Blepharisma undulans* by U.V.[10] and change in discharge from a pressure receptor of the crayfish produced by U.V.[11], have been reported as failing to show photoreversal. Drastic action of U.V. is not subject to photoreversal[6].

The failure of photoreactivation in *Hemophilus influenzae* has been brilliantly exploited by investigators at Johns Hopkins University[9] as was so clearly described by Dr. Rupert at the present symposium. Failure to obtain photoreactivation in this case is the consequence of lack of the appropriate photoenzyme in *Hemophilus*; not, however, of the other constituents, since photoreactivation of *Hemophilus* transformation is readily achieved with extracts of *Escherichia coli* or yeast containing the photoenzyme. It therefore becomes important that other species which fail to show photoreactivation be subjected where possible to a similar analysis.

Failure to obtain photoreactivation in some yeast mutants has been shown at the present symposium by Dr. Pittmann to result from lack of appropriate hereditary determinants. Such non-photoreversible mutants may be tested by the Rupert-Herriott transforming-principle technique as described at the symposium.

The retardation of division by U.V. of *Blepharisma undulans*, a large pink ciliate, shows an action spectrum suggestive of the absorption spectrum of a nucleoprotein[10]. No photoreactivation was observed when the U.V.-treated animals were illuminated with light from fluorescent lamps which for the same dosage produced maximal photoreactivation in other protozoans and yeast. Since *Blepharisma* contains a pink pigment in granules located in the pellicle and since the absorption spectrum of the pigment is considerable in the regions of the spectrum most effective in photoreactivation[12], it seemed likely that visible light simply could not reach the site of photoreactivation or did so to a negligible degree.

The pigment of *Blepharisma* is photodynamic and strong visible light kills the cells[12]. Weak light, however, does not kill the cells but they divide at a slower rate. An alternative explanation to that previously presented for the failure to get photoreversal in this species is that the light reaches the site of photoreversal but that photoreversal is counteracted by the damaging photodynamic action of the light. The damaging effect of light should disappear in the absence of oxygen as in all true photodynamic effects. This proved to be true when the oxygen tension was sufficiently reduced and under these conditions photoreactivation was obtained in all cases tested. When sufficiently low intensities and short dosages of light were used it was even

References p. 322

possible to demonstrate photoreversal in *Blepharisma* when oxygen was present, although the results are not quite as striking as in its absence[13]. The results on *Blepharisma* therefore suggest a further test that should be made on so-called photoreversal-refractory organisms. Some findings on bacteria suggest a similar explanation (ref.[6], p. 106). Oxygen is not necessary for photoreactivation[14].

In closing the symposium I should like to thank all the speakers for their able presentations and the audience for the stimulating discussions which have added much interest and zest to the meeting. Particularly gratifying has been the opportunity which this symposium has offered for the meeting of investigators from many lands, with the resultant stimulation and the greater understanding that has developed between us all.

REFERENCES

1 R. L. AMY AND R. C. VON BORSTEL, *2nd Intern. Congress on Photobiology, Turin 1957*, Minerva Medica, Turin, 1958.
2 R. C. VON BORSTEL AND S. WOLFF, *Proc. Natl. Acad. Sci. U.S.*, 41 (1955) 1004.
3 C. L. BRANDT AND A. C. GIESE, *J. Gen. Physiol.*, 39 (1956) 735.
4 S. PIERCE AND A. C. GIESE, *J. Cellular Comp. Physiol.*, 49 (1957) 303.
5 J. BOOTH, A. VON MURALT AND R. STÄMPFLI, *Helv. Physiol. Pharmacol. Acta*, 8 (1950) 110.
6 J. JAGGER, *Bacteriol. Rev.*, 22 (1958) 99.
7 W. D. BELLAMY AND M. T. GERMAIN, *J. Bacteriol.*, 70 (1955) 351.
8 C. R. GOUCHER, I. KAMEI AND W. KOCHOLATY, *J. Bacteriol.*, 72 (1956) 184.
9 S. H. GOODGAL, C. S. RUPERT AND R. M. HERRIOTT, IN W. D. MCELROY AND B. GLASS, (editors), *The Chemical Basis of Heredity*, The Johns Hopkins Press, Baltimore, 1957
10 H. HIRSHFIELD AND A. C. GIESE, *Exptl. Cell Research*, 4 (1953) 263.
11 A. C. GIESE AND E. J. FURSHPAN, *J. Cellular Comp. Physiol.*, 44 (1954) 191.
12 A. C. GIESE, *J. Gen. Physiol.*, 37 (1953) 259.
13 A. C. GIESE AND M. LUSIGNAN, *J. Gen. Physiol.*, 44 (1961) 543.
14 F. H. JOHNSON, E. A. FLAGLER AND H. F. BLUM, *Proc. Soc. Exptl. Biol. Med.*, 74 (1950) 32.

Symposium 7

PHOTOTHERAPY

Chairman: JEAN MEYER, Paris (France)

Secretary: EVA CHIEVITZ, Copenhagen (Denmark)

LICHTBEHANDLUNG VON HAUTKRANKHEITEN

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Die Lichttherapie der Hautkrankheiten ist eine Erfahrungswissenschaft. Zwar wurden zahlreiche Erkenntnisse über die biologischen Wirkungen des Lichtes auf die Zelle, die Haut und den Gesamtorganismus gewonnen. Ein direkter Zusammenhang zwischen biologischem und therapeutischem Effekt kann jedoch häufig nur vermutet werden.

Eindeutig ist der Zusammenhang bei der Rachitis. Vom Organismus selbst synthetisiertes 7-Dehydrocholesterin geht unter Einwirkung kurz- und mittelwelligen U.V.-Lichtes in antirachitisch wirksames Vitamin D_3 über. Nach van der Lugt und Rottier[1] ist die Vitamin D_3-bildende Strahlung < 310 mμ auch für den therapeutischen Effekt des Lichtes bei der Hauttuberkulose verantwortlich zu machen. Bei der Psoriasis kann eine gleichartige Wirkungsweise nur vermutet werden. Hier ist eine therapeutische Wirkung auch über die Photooxydation von Sulfhydrilgruppen denkbar. Solange wir aber nichts Näheres über die der Psoriasis zugrundliegende Stoffwechselstörung der Haut wissen, bleiben die genannten Vermutungen reine Spekulation.

Die den stärkeren Graden der Lichtentzündung nachfolgende Hautschälung dürfte für den therapeutischen Effekt des ultravioletten Lichtes bei der Pityriasis rosea und versicolor verantwortlich sein. Bei der Akne vulgaris kann man ebenfalls den hautschälenden Effekt nutzen. Wirksam sind aber auch unterhalb der Erythemschwelle liegende Lichtdosen. Hier wie bei den bakteriell entzündlichen Hauterscheinungen einschliesslich des bakteriellen und seborrhoischen Ekzems dürften dem therapeutischen Effekt die vielfältigen Wirkungen des Lichtes auf den Gesamtorganismus und seine Immunitätslage, insbesondere über die Freisetzung von Zellabbauprodukten, zugrundeliegen.

Bei der Beeinflussung der Ichthyosis durch Sonnenlicht ist eine Umwandlung von Hautcarotin in Vitamin A ähnlich einem analogen Vorgang in der Netzhaut denkbar.

Die Wirkungsspektren der zugrundeliegenden biologischen Lichtreaktionen geben Hinweise für die therapeutisch einzusetzenden Spektren. Die Auswahl ist begrenzt. Die Quecksilberhochdrucklampe hat sich gegenüber den Kohlenbogenlampen immer mehr durchgesetzt. Variationsmöglichkeiten liegen in der Abfilterung des kurzwelligen oder des gesamten erythemerzeugenden Anteils des Quecksilberspektrums und in der Kombination mit Infrarotlicht.

Xenonhochdrucklampen haben sich in der Therapie wegen des grossen technischen Aufwandes nicht durchgesetzt. Ihre kontinuierliche Sonnen-ähnliche spektrale Energieverteilung im U.V.- und sichtbaren Bereich ist mehr von lichtbiologischem als von therapeutischem Interesse. Die von uns benutzte Type vermag zwar in 20 Min ohne Erythem direkt zu pigmentieren. Andere photochemische Wirkungen des

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Literatur S. 328

langwelligen U.V.-Lichtes auf die Haut sind jedoch bis heute nicht bekannt geworden.

Eine neuartige Bestrahlungslampe stellt eine Fluoreszenzleuchte mit kontinuierlichem Emissionsspektrum im U.V. dar. Das Emissionsmaximum liegt übereinstimmend mit dem Maximum der spektralen Erythemwirksamkeit des Sonnenlichtes bei 305 mμ. Leistungsaufnahme und Abmessungen entsprechen denen der üblichen Beleuchtungslampen. Es können also auch die üblichen Fassungen, Starter etc. verwendet werden. Nach den bisher vorliegenden eigenen Messungen und Erfahrungen ist die Erythemwirksamkeit gering. Bei der von uns ausgewählten Lampenanordnung mit Aluminiumreflektoren wurden bei 35 cm Lampen-Hautabstand für eine Erythemschwellenzeit von 1 Min 8 40-W Lampen benötigt. Abgesehen von der fehlenden Einbrennzeit, dem geringen Stromverbrauch und der langen Lebensdauer erhoffen wir uns einen Vorteil bezüglich der Feldausleuchtung bei der Ganzbestrahlung, z.B. von Psoriasis-Patienten. Bisher haben wir mehrere innen verspiegelte U.V.-Infrarot-Glaskolbenlampen so angeordnet, dass bei einer anfänglichen Erythemschwellenzeit von 3–5 Min die ganze Körperoberfläche annähernd gleichmässig ausgeleuchtet wurde. Bei einer Steigerung der Bestrahlungszeit um 10% von Bestrahlung zu Bestrahlung wurde maximal 20 Min lang bestrahlt. Eine Verringerung des Lampenabstandes von der Haut zur Einsparung von Bestrahlungszeit ist wegen der gleichzeitigen erheblichen Wärmebelastung nicht möglich. Hier setzt der Vorteil des kalten Fluoreszenzlichtes ein, mit dessen Hilfe wir bei gleichem Gewöhnungsfaktor und damit gleicher Dosissteigerung auf Bestrahlungszeiten von einer bis maximal 5 Min zu kommen hoffen.

TABELLE I

RELATIVE DURCHBLUTUNGSGRÖSSEN DER HAUT NACH 20 MIN BESTRAHLUNG BEI GERADE NOCH VERTRÄGLICHER BESTRAHLUNGSSTÄRKE

	Glühlampe	*Glühlampe mit Wasserfilter*	*Heizdraht*
Rel. Bestrahlungsstärke	1	1.33	0.54
	1	1.02	0.8
	1	0.84	0.75
	1	1	0.77
	1	1.04	1.02
	1	1.27	0.8
	1	0.89	0.82
	1	1.13	1.21
	1	1	1.04
	1	1.25	1.1
	1	0.96	0.76
Mittelwert	1	1.04	0.907

Die Dosierung der U.V.-Strahlung erfolgt nach wie vor auf der Basis der entzündlichen U.V.-Reaktion. Der Vorteil liegt in der Vermeidung schwerer Verbrennungen, der Nachteil in dem nicht immer gegebenen Zusammenhang zwischen entzündlicher und erwünschter therapeutischer Reaktion. Die Bestimmung der Erythemschwelle vor jeder Bestrahlungsserie erfasst wohl Veränderungen der Dosisleistung, z.B. infolge Alterung des Brenners und individuelle Empfindlichkeitsunterschiede, verliert jedoch an Wert bei regional unterschiedlicner Vorbestrahlung. In der Regel dürften Arzt und Patient zur routinemässigen Erythemschwellenbestimmung nicht die genügende Zeit aufbringen.

TABELLE II

ZUORDNUNG DERMATOLOGISCHER INDIKATIONEN ZU OPTIMAL WIRKSAMEN LAMPENTYPEN*

Diagnose		Bestrahlung ganz	Bestrahlung lokal	Optimaler Erythemgrad	Hg-Hochdrucklampen Ungefiltert	Hg-Hochdrucklampen Typ Ultravitalux	Hg-Hochdrucklampen Kromayer	Kohlenbogen	Sonne	Infrarot	U.V.-bestrahltes Eigenblut
Gesicherte Indikationen											
Acne vulgaris	(1)		+	mittelstark—stark	+						
	(2)	+	+	unterschwellig—schwach	+	+		+	+		
Acne indurata			+	mittelstark—stark	+		+				
Psoriasis general		+		schwach	.	+		+	+		+
Psoriasis Plaques			+	mittelstark—stark			+				
Parapsoriasis		+		mittelstark	+						
Mycosis fungoides (praemykotisches und infiltratives Stadium)		+		unterschwellig-schwach					+		
Pityriasis rosea		+		schwach	+						
Pityriasis versicol		+		mittelstark	+						
Bakt. u. seborrh. Ekzem		+		unterschwellig—schwach	+	+		+	+		+
Furunkulose		+		unterschwellig—schwach	+	+				+ (lokal)	+
Hidradenitis			+							+	
Trichophytia prof.			+							+	
Follikulitis barbae			+	unterschwellig—schwach	+	+					
Ichthyosis		+		unterschwellig—schwach				+	+		
Hauttuberkulose	(1)	+		unterschwellig—schwach		+		+	+		
	(2)		+	sehr stark			+	+ (gefilt.)			
Unzureichend gesicherte Indikationen											
Alopecia areata			+	stark—sehr stark	+		+				
Alopecia seborrh.			+	schwach	+		+				
Zoster Neuralgien		zugehöriges Spinalganglion		mittelstark	+					+	
Lichen ruber plan.		+		unterschwellig—mittelstark	+						
Lichen ruber verr.			+	stark—sehr stark	+		+				
Pruritus		+		unterschwellig	+	+		+	+		
Torpide Ulcera			+	unterschwellig—schwach	+	+		+	+		
Vitiligo			+	vorsichtige Photosensibilisierung	(+)	(+)	(+)	+	+		

* Aus *Handbuch der Haut- und G. Krankheiten* V/2 Springer, Berlin, 1959 (Beitrag Kimmig und Wiskemann).

Literatur S. 328

Für die therapeutische Wirkung der Infrarotstrahlung auf Zoster-Neuralgien und lokale Entzündungen dürfte die reaktive Durchblutungssteigerung entscheidend sein. Im Rahmen der gegenregulatorischen Wärmeabgabe kommt es zu einer gesteigerten Hautdurchblutung. Die Impulse hierzu gehen von der 0.1 mm unter der Hautoberfläche gelegenen Schicht der Thermorezeptoren aus. Entscheidend für die Temperatur und Schmerzempfindung und damit für die Thermoregulation ist nach Hensel[2] allein die Temperatur in dieser Hautschichttiefe.

Wenn dem so ist, so müssten spektral unterschiedliche Infrarotstrahler bei gleicher, durch die Temperatur am Orte der Thermorezeptoren bestimmter Schwelle der Schmerzempfindung eine gleichstarke Durchblutungssteigerung der Haut bewirken. In der Tat konnten wir nach 20 Min Bestrahlung mit einer hochbelasteten Wolframglühlampe, einer gleichartigen Lampe mit Wasserfilter und einem Heizdraht bei unterschiedlicher, aber jeweils gerade noch verträglicher Bestrahlungsstärke nahezu gleiche Hautdurchblutungsgrössen messen (Tabelle I).

Als Mass der Hautdurchblutung wurde die Scheinleitfähigkeit der Haut mit Hilfe eines, nach Angaben von Hensel[3] konstruierten Gerätes* bestimmt.

Wenn insbesondere die reaktive Steigerung der Hautdurchblutung einschliesslich der konsensuellen Durchblutungssteigerung und weniger die passive Erwärmung des Gewebes (durch Absorption und Wärmeleitung) zum therapeutischen Effekt beiträgt, dann dürfte es ziemlich gleich sein, ob grössere Energien des stärker penetrierenden kurzwelligen Infrarotlichtes oder geringere Energien des weniger penetrierenden mittel- bis langwelligen Infrarotlichts in die Haut eingestrahlt werden, sofern der Abstand der Infrarotquelle von der Haut durch die maximal verträgliche Dosisleistung bestimmt wird.

Zum Schluss möchte ich kurz auf den durch die Schaffung überlegener Heilverfahren zunehmend eingeschränkten Indikationsbereich der dermatologischen Lichttherapie eingehen. Als gesicherte und auch heute noch gültige U.V.-Indikationen gelten die Akne, Psoriasis, Parapsoriasis, Mycosis fungoides[4], Pityriasis rosea und versicolor, Furunkulose, resistente Fälle von Folliculitis barbae, bakterielles und seborrhoisches Ekzem, die Ichthyosis und -- weitgehend durch die Isonikotinsäurehydrazid-Therapie verdrängt -- die Hauttuberkulose. Unzureichend gesichert sind die Erfolge bei Alopecia areata, Zoster-Neuralgien, Lichen ruber, Pruritus, Ulcera und Vitiligo. Als gesicherte Indikationen der Infrarot-Therapie müssen die Hidradenitis, die tiefe Trichophytie und allenfalls Zoster-Neuralgien und Unterschenkelgeschwüre angesehen werden.

Den auf den eingangs zitierten Überlegungen und klinischer Erfahrung fussenden Versuch einer Zuordnung dermatologischer Indikationen zu optimal wirksamen Lampentypen und Erythemgraden mögen Sie Tabelle II entnehmen.

Mit Rücksicht auf das vor uns liegende grosse Programm möchte ich mein Referat beenden. Es sollte Sie kurz über die biologischen Grundlagen, den technischen Stand, und die zur Zeit gültigen Indikationen der Lichttherapie von Hautkrankheiten informieren.

LITERATUR

1 L. VAN DER LUGT UND P. B. ROTTIER, *Acta Dermato-Venereol.*, 38 (1958) 264.
2 H. HENSEL, *Arch. ges. Physiol., Pflüger's*, 252 (1950) 165.
3 H. HENSEL UND F. BENDER, *Arch. ges. Physiol., Pflüger's*, 263 (1956) 603.
4 K. LINSER, *Z. Haut und G. Krankh.*, 27 (1959) 319.

* Fluvograph der Firma Hartmann und Braun, Frankfurt.

THE VASCULAR REACTION OF HUMAN SKIN TO IRRADIATION WITH LONG WAVE ULTRAVIOLET (366 mμ) AND VISIBLE LIGHT (405 AND 436 mμ)

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In an attempt to reproduce the "immediate pigmentation" which is said to require doses of long wave U.V. which are 500–1000 times as heavy as those which cause the normal sunburn reaction with short wave U.V., the light of a strong mercury arc (SP 500, Philips) was focused on the skin by glass lenses after passage through interference filters. Measures had to be taken to prevent any short wave U.V. reaching the skin.

The apparatus[1] used realizes during an irradiation of 1 min doses of 366, 405 and 436 mμ of about 1000 times the short-wave sunburn dose, which amounts on the upper arm to about 25 mWsec/cm^2. During an irradiation of 1 min the contamination with short-wave ultraviolet is less than 0.0001 of a sunburn dose.

The reaction of the skin (mostly of the upper arm) of about 40 patients was studied with the three wave lengths mentioned, and with irradiation times up to 20 min (doses up to 300 Wsec/cm^2). In most cases there were visible reactions which can be summarised under the following three heads:

1st: in about 80% of the cases an erythema was visible immediately after the irradiation. It lasted for not longer than 3–5 min and had no after-effects.

2nd: in about 20% an erythema appeared, after a latent period of several hours. It somewhat resembled the normal sunburn reaction.

3rd: in about 70% a bluish-grey discolouration was visible immediately after the irradiation lasting for not longer than a few hours. It was not "immediate pigmentation" as it disappeared completely under pressure from a piece of glass or plastic. Later, when the original bluish-grey had diminished, a faint yellowish or brownish colour remained visible. Some days later, if sufficient irradiation had been given, it was succeeded by pigmentation. If the skin was treated with an aqueous solution of 2% naphazoline[2] (a constrictor of precapillary blood vessels) + 2% sulfas atropini (which strongly fortifies the action of naphazoline), development of the bluish-grey discolouration was prevented. We refer to this 3rd reaction as "blue hyperaemia" of the deeper vessels of the skin. The faint discolouration when it was under pressure or when it had disappeared was presumably caused by cellular infiltrations around the vessels involved.

The three types of visible reaction seem to be independent. They can be present

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References p. 330

together and can succeed one another. In most cases the blue hyperaemia or the late erythema were observed whether or not preceded by the early erythema. In some cases the 2nd or 3rd reaction was accompanied by oedema.

We have come to the conclusion that all primary visible skin reactions caused by irradiation with U.V. or visible light are vascular dilations, the red ones more superficial, the blue ones deeper in the skin. It is apparent that the vascular behaviour of different skins may be quite different.

REFERENCES

[1] P. B. Rottier and J. C. van der Leun, *Brit. J. Dermatol.*, 72 (1960) 256.
[2] R. W. von Nordheim, *Ophthalmologica*, 132 (1956) 327.

THE NORMAL SENSITIVITY OF HUMAN SKIN TO 250 AND 300 mμ ULTRAVIOLET IRRADIATION AND ITS DEPENDENCE ON THE THICKNESS OF THE STR. CORNEUM

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An approximate minimum erythema dose (MED) for the wave length regions of 300 and 250 mμ, to which the skin is particularly sensitive, was determined on the upper arms of 149 in-patients who did not suffer from light dermatoses, and who were selected for reliable results.

The quartz spectrograph and mercury lamp used for the irradiation had the advantage of giving an almost fixed ratio for the intensities of both wavelengths, while the energy falling on the skin had always photometrically been brought to the same level before each irradiation. Fig. 1 shows the mean MED's for all persons expressed in seconds irradiation time, the two mean monthly MED's for the months June 1958 to April 1959, the mean of the total, and the monthly ratio MED 250/MED 300.

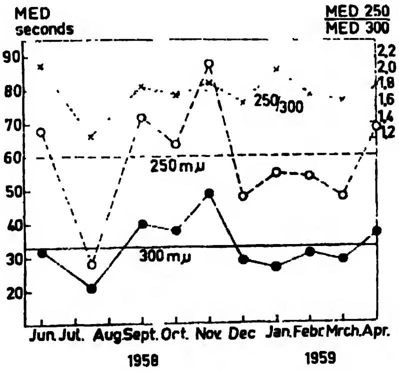

Fig. 1. Total and monthly means of the MED 300, of the MED 250 and of the ratio MED 250/MED 300 on the upper arm of 149 persons. A mean MED 300 of 33 sec corresponds with approximately 25 mWsec/cm^2.

From Fig. 1 the mean sensitivity of the patients would seem to be highest during July-August and lowest during September-November (perhaps also during April-May). The lowest sensitivity seems to occur in those periods when most light dermatosis cases present themselves at the clinic. Moreover, it will be seen that an increase in the MED 300 is accompanied by an increase in the MED 250 and also by

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References p. 332

an increase in the ratio MED 250/MED 300. This ratio tends to be high when the mean sensitivity is low. This dependence of the ratio 250/300 on sensitivity points to the fact that the screening power of the horny layer which determines the overall sensitivity of the skin is greater for 250 mμ than for 300 mμ.

An approximate calculation of the dependence of the two MED's and their ratio has been carried out in the following way. Using several extinction coefficients of the horny layer at 300 and 250 mμ, the MED's were calculated for several thicknesses of this layer on the upper arm. The results were compared with a plot of these values as they were determined for the 149 patients examined. The best fit gave the values in Table I. Note that in this table the figures are given in different units (mWsec/cm^2) from those in the figure (seconds irradiation time with our apparatus).

TABLE I

Thickness of str. corneum μ	*MED 300 mWsec/cm^2 epicorneal*	*Ratio 250/300*
0	3.3	0.25
7	6.6	0.36
14	13.3	0.52
20	24.0	0.70
27	48	1.00
35	106	1.50
42	212	2.14
49	425	3.03

With the help of the values in this table more accurate determinations of the MED's will be possible, which in turn will permit calculation of more accurate values for the dependence of the ratio 250/300 on the thickness of the horny layer. The values given in Table I have already made it possible to distinctly separate abnormal MED's from those which were apparently determined with a low accuracy. In this way the normal MED 300 on the upper arm was established to be 24 mWsec/cm^2 for women (ratio 0.7) and 19 mWsec/cm^2 for men (ratio 0.6).

The normal thickness of the horny layer on the upper arm varies over the range 8–27 μ. An increase of this thickness from 20 to 40 μ decreases the sensitivity for 300 mμ to about 1/8 of the normal sensitivity. This increase can easily be obtained in normal persons by irradiation with U.V. light. If it can be obtained in light dermatoses it will be more effective than, and preferable to, most screening mixtures.

Correctly determined abnormal MED's which show a ratio 250/300 quite different from those given in the table point to abnormal erythematogenous processes in the skin. This has been found in several cases and indicates independence of the two processes[1,2].

REFERENCES

[1] P. B. Rottier, *J. Clin. Invest.*, 32 (1953) 681.
[2] P. B. Rottier, *Proc. 1st Intern. Congress Photobiol., Amsterdam*, 1954, p. 192.

DRUG-RESISTANT MALARIA TERTIANA CURED BY PHOTOTHERAPY

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Although phototherapy is not a new method of treating malaria — in 1936 C. Wyckerheld Bisdom and D. Mulder reported cases of malaria cured by phototherapy at the Third International Light-Congress — I think it as well to present to you the history of a cure of a case of malaria tertiana which I was in a position to follow very closely. In fact, the patient happened to be myself.

When 25 years old, the patient went to the tropics as a soldier — his medical examination had shown him to be in good health. On board the troopship the customary quinine prophylaxis against malaria was started, and continued during the following years in the tropics. In October 1947, after one year in the tropics and shortly after transference to a highly malarious area, the first symptoms of malaria manifested themselves: headache, shaking chills, fever. Laboratory tests for malaria were however, negative. In December 1947 the same symptoms recurred; the patient was admitted to hospital with high fever and this time malarial parasites were found in the bloodsmear: malaria tertiana. Therapy was started at once with quinine and plasmochine. Discharge from the hospital followed after 7 weeks, by which time the bloodsmears were repeatedly negative for malaria. A relapse occurred in April 1948 and the patient was rehospitalized with high fever. The therapy with quinine and plasmochine was supplemented with atebrine, and the patient was discharged after 7 weeks, once again after repeatedly negative blood tests. Subsequently paludrine (which was then new) was used as a prophylactic measure, alternating with atebrine.

On his return to the Netherlands in June 1949, the patient was examined and found to be anaemic, too thin (weight 65 kg, height 1.82 m) and enfeebled. This was attributed to malaria which was latent as a result of the drug treatment. The physician predicted relapse if prophylaxis was discontinued. This had already been done and symptoms of malaria did indeed appear at the end of June 1949 in the form of general malaise, fever and shaking chills. The bloodsmears were highly positive for malaria tertiana.

The patient was now left with the alternatives of continuing the chemotherapy or changing to general phototherapy. Since the former had failed to effect a permanent cure in the preceding years, the patient chose the latter course. General phototherapy should be taken to mean irradiation with ultraviolet and infrared light, the light sources being at such a distance from the patient that he receives as it were a "lightbath". The dosage is gradually increased in proportion to the needs and tolerance of the patient.

To gain a clearer view, one of the walls of a small therapy room was removed. This room measures approximately $2\frac{1}{2} \times 2\frac{1}{2} \times 2\frac{3}{4}$ m, and is provided with 6 Sollux (infrared) lamps of 1000 watts each and 6 mercury vapour lamps (ultraviolet) of 700

watts each. The patient lies wholly or partially undressed on a low couch or on a mattress on the floor, so that the distance from the light source is about 2 m (Fig. 1). In this way a diffuse exposure is brought about with ultraviolet. The infrared lamps yield a more direct radiating warmth which is enhanced by their reflectors.

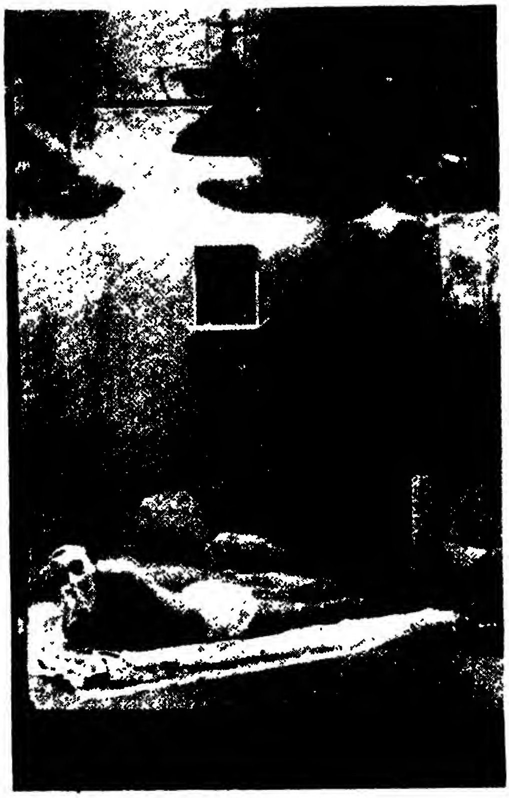

Fig. 1.

The period of treatment by phototherapy for the permanent cure of this malarial infection lasted from the end of June until mid-August. Fig. 2 shows the temperature curve for this period. It shows 4 groups of regular tertian attacks of fever alternating with a few days of normal temperature; there were 21 attacks in all. The arrows at the top of the graph indicate the number of lightbaths received in this period, a total of 46. The intensity and duration of the exposures was gradually increased in accordance with the patients endurance, *i.e.* from about 5 min at the start of the treatment to 30–45 min at its height.

The attacks were mostly severe, but did at length abate. They began with severe headaches, nausea and vomiting followed by shaking chills, although their unpleasantness was greatly mitigated by the beneficial warmth of the infrared lamps. Soon after the chills the temperature rose quickly (highest temperature recorded, 41.7°) and persisted for some hours; a cold shower could then make the temperature drop fairly rapidly to more normal values. During the afebrile days and periods the patient was up, did his work, walked, in short was able to lead a practically normal life. Because of this, when the attacks ceased, convalescence was quite rapid.

Table I shows some laboratory findings on the bloodsamples taken during and after

the phototherapy. It should be noted that this examination of the blood reflects more accurately the actual situation than that during and after chemotherapy, since the malarial plasmodia do not retreat from the peripheral blood and lodge in organs such as the spleen and liver.

The notable fact here therefore, is that a malarial infection which had already become chronic, which had resisted prolonged therapy with the known antimalarial agents and had become resistant, was permanently cured. Up till the present moment,

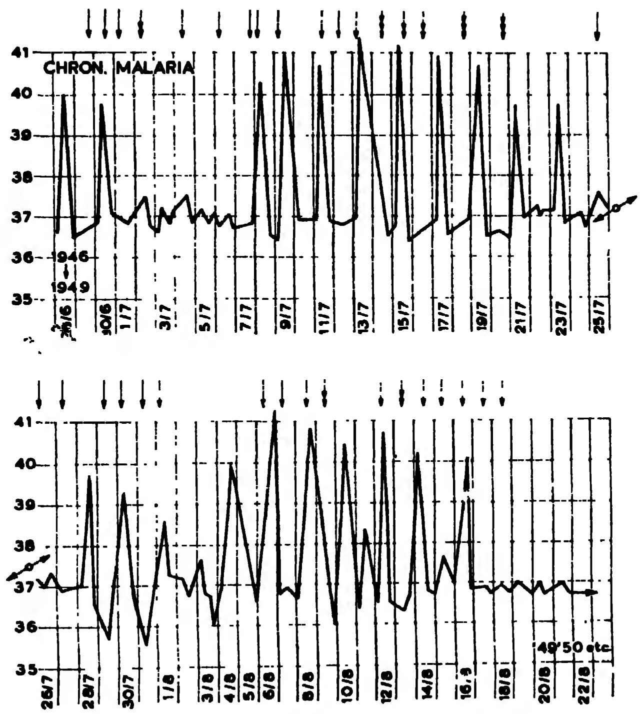

Fig. 2.

TABLE I

WEIGHT OF PATIENT AND BLOOD ANALYSES DURING AND AFTER PHOTOTHERAPY

Year	1949						1950
Date	*30/6*	*27/7*	*30/8*	*10/9*	*1/10*	*31/10*	*10/8*
Weight (kg)	65	65.8	66.5	71	71	71	
Erythrocytes (mill.)	4.5	2.9	3.68	3.85	4.74	4.28	4.47
Hemoglobin (%)	90	65	75	77	95	85	91
Slide (malaria)	+++	++	++	+	—		—

11 years later, the patient has remained completely free from complaints which might be attributed to malaria.

The difference from the usual course of treatment lay in the fact that no drugs were prescribed or taken, that only optimal daylight was used, that bed-rest proved necessary only during attacks and that a complete and permanent recovery was affected rather than a rapid, but impermanent cure.

DIE BEDEUTUNG VON LATENZZEITEN FÜR U.V.-STRAHLEN-BEDINGTE VERÄNDERUNGEN AM LEBENDEN GEWEBE*

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Primäreffekte, die durch ultraviolette Strahlen hervorgerufen werden, können nur in den obersten Hautschichten erwartet werden, da die Eindringtiefe dieser Strahlen nicht sehr gross ist. In früheren Untersuchungen (Bücker und Hanke[1]) konnte geklärt werden, dass bereits bei einer Eindringtiefe von etwa 30 μ die sehr wirksame Strahlung des U.V. B (280–320 mμ) im Durchschnitt auf etwa 30% geschwächt wird. Dadurch beträgt die an dieser Stelle absorbierte Energie nur noch etwa 1% der auftreffenden Strahlung.

In diesen oberen Hautschichten treten auch bereits Sekundäreffekte auf, die von den Primäreffekten angeregt werden und nach einer bestimmten Latenzzeit nachweisbar sind. Auch sie können für den Körper eine Reizquelle darstellen. Primär- und Sekundär-Reaktionen in der Haut lassen sich zum Teil histochemisch nachweisen. Diese Reaktionen spiegeln Zellveränderungen wieder, die teilweise reversibel, teils aber irreversibel sind. Im letzten Falle haben diese Veränderungen dann als Sekundärreaktionen die Ausbildung von Regenerationsblastemen zur Folge, die sich histochemisch durch besondere Aktivität auszeichnen.

METHODIK

Der Nachweis der Veränderungen erfolgte an Querschnitten von Häuten, bei denen eine kleine Stelle bestrahlt worden war. Die Strahlung wurde streng lokalisiert, so dass im Querschnitt neben der bestrahlten Stelle unbestrahlte Regionen zur Kontrolle zur Verfügung standen. Die Bestrahlung erfolgte in der Regel am Tier; die Feststellung der Veränderung entweder sofort nach der Bestrahlung oder nach Latenzzeiten, wenn die Untersuchung von Sekundärreaktionen geplant war. Die Bestrahlungen wurden an Rückenhautbezirken von weissen Mäusen und Froschschwimmhäuten vorgenommen. In beiden Fällen war gewährleistet, dass nur eine dünne Hornhaut vorlag, so dass keine starke Absorption des kurzwelligen U.V. angenommen werden musste.

Als Bestrahlungsquelle dienten Quecksilberhochdruckbrenner. Die angewandten Intensitäten sind in früheren Arbeiten angegeben (Hanke[2-4]). Die eingestrahlte Dosis bei Bestrahlungszeiten von 5–30 min liegt wesentlich über der Erythemdosis (im Durchschnitt etwa das 10-fache derselben). Diese hohen Intensitäten wurden ange-

* Meinem verehrten Lehrer, Herrn Prof. Dr. H. Giersberg zum 70. Geburtstag gewidmet.
** Direktor Prof. Dr. H. Giersberg.

wandt, da die Reaktionen auch noch in tieferen Hautschichten deutlich werden sollten. Aus diesem Grunde sind die Veränderungen bei therapeutischer Anwendung der U.V.-Strahlen nicht in diesem Ausmass zu erwarten. Es ist aber ohne weiteres anzunehmen, dass auch bei Bestrahlungsdosen in der Grössenordnung der Erythemschwelle entsprechende Zellveränderungen in der Epidermis auftreten, die als Reiz für den Körper gelten können. Die Untersuchungen sollen also als Modellversuche verstanden werden, aus denen sich die Veränderungen nach therapeutischen Bestrahlungsdosen abschätzen lassen. Es handelt sich hierbei um die Erforschung der grundsätzlichen Zellreaktionen auf Bestrahlung hin. Gewisse unterschiedliche Reaktionen treten bei Zellen verschiedenen Differenzierungsgrades auf, da diese sich in Bezug auf die Ausgangssituation beachtlich unterscheiden können.

ERGEBNISSE

Die auf Bestrahlung beobachteten Reaktionen sind sehr vielgestaltig. Oxydationswirkungen, die sich als Aktivitätssteigerung des Peroxyd–Peroxydase-Systems und der Tyrosinase nachweisen lassen, treten im bestrahlten Bereich auf (Dubouloz und Dumas[5]; Hanke[2,6]). Sie werden mit Hilfe der Nadi-Reaktion am unfixierten und der Dopa-Reaktion am formolfixierten Querschnitt deutlich. Durch Katalase, die Peroxyde in der Haut zerstört, wird die Nadi-Reaktion verhindert. Hieraus ergibt sich die Bedeutung der gebildeten Peroxyde für diesen Prozess. Bei einer Reihe von Dehydrogenasen konnten Inaktivierungen beobachtet werden (DPN-Diaphorase, Bernsteinsäuredehydrogenase und Milchsäuredehydrogenase). Damit dürfte nachgewiesen sein, dass der Atmungsstoffwechsel der Zelle stark gestört ist (Hanke[3]). An zwei weiteren Fermentsystemen konnten Strahlungsveränderungen deutlich gemacht werden. Sowohl die Aktivität von alkalischer Phosphatase als auch von unspezifischer Esterase wurde verringert (Hanke[4]). Diese Fermente sind in den Differenzierungsvorgang eingeschaltet. Sie dienen wohl weitgehend dem Nukleinsäurestoffwechsel und dem Aufbau spezifischer Zellverbindungen, die für besonders differenzierte Zellen von Bedeutung sind. Der Angriff der ultravioletten Strahlen auf den Nukleinsäuregehalt zeigt sich aber noch deutlicher. Durch eingehende Untersuchungen über den strahleninduzierten Kernzerfall ist klar geworden, dass die DNS des Kernes Angriffspunkt der Strahlung ist und durch kurzwelliges U.V. stark verändert wird. Neben der DNS zeigt aber auch die RNS des Cytoplasmas Reaktionen auf Bestrahlung. Der Nachweis der RNS in den Zellen erfolgt mittels gepufferter Methylenblau-Lösung (pH 5.0). Bei diesem pH beruht die Basophilie des Cytoplasmas fast nur auf RNS, wie sich durch vorheriges Inkubieren der Schnitte in Ribonuklease leicht nachweisen lässt. Direkt nach der Bestrahlung ist die Basophilie des Cytoplasmas im allgemeinen erhöht. Dies deutet jedoch nicht auf eine Erhöhung der Menge von RNS hin, sondern beruht auf einer Vermehrung freier basophiler (saurer) Gruppen. Nach einiger Zeit wird diese veränderte RNS abgebaut. Die bestrahlte Stelle wird weitgehend frei von RNS. Es erfolgt dann erneuter Aufbau über das Regenerationsblastem. Zur Unterscheidung von Primär- und Sekundär-Reaktionen ist die Beachtung von Latenzzeiten bis zum Feststellen des Erfolges von grosser Bedeutung. Tritt eine Reaktion erst nach Verlauf einiger Stunden auf, so kann als sicher angenommen werden, dass sie sich aus der Anstoss-Reaktion erst entwickeln musste, unter Umständen mit Hilfe verschiedener Systeme. Andererseits ist eine strenge Trennung der Reaktionen, die im Strahlenabsorptionsgebiet ablaufen, wahrscheinlich nicht so einfach möglich.

Literatur S. 341

Häufig konnten Reaktionen erst nachgewiesen werden, wenn die am Tier bestrahlte Hautstelle noch eine bestimmte Latenzzeit lang am intakten Tier verblieb. Die Einflüsse von Latenzzeiten zeigten sich aber auch dann, wenn Wirkungen schon direkt nach der Bestrahlung beobachtbar waren. Kleinere Dosen, die noch keine Reaktion direkt nach der Bestrahlung nachweisbar werden liessen, zeitigten eine solche nach einer Latenzzeit, wenn das Hautstück am intakten Tier verblieb.

Die Notwendigkeit einer Latenzzeit ergibt sich aus folgenden Punkten:

(1) Die Blutversorgung muss bestimmte Stoffe zum Bestrahlungsort bringen und eine hohe Sauerstoffspannung dort aufrecht erhalten.

(2) Der Abbau primär veränderter Stoffe bedarf einer gewissen Zeit.

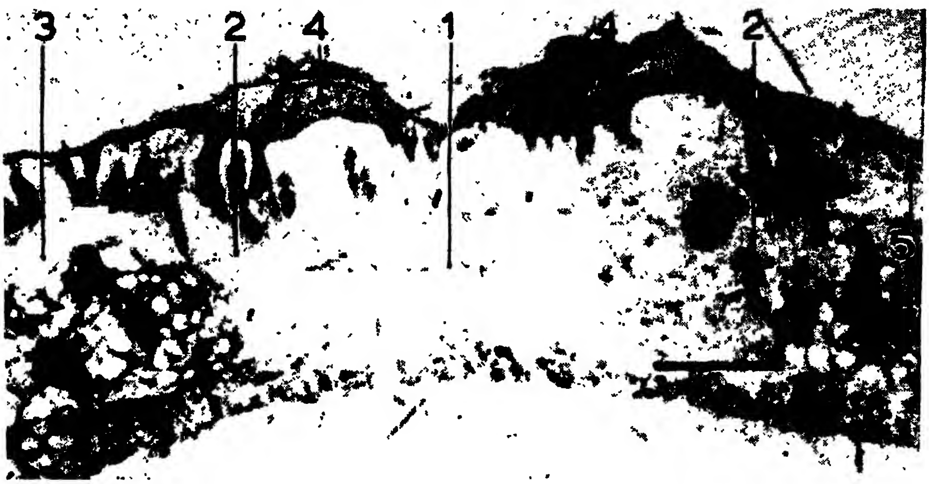

Abb. 1. Mäuserückenhaut, quer, Gefrierschnitt, unfixiert. Vers. M 34, Bestrahlungszeit 30 min, Latenzzeit 196 Std., DPN-Diaphorase-Nachweis, Vergr. 35-fach. 1 Zentrum der bestrahlten Stelle, Inaktivierung des Fermentes, 2 Regenerierende Grenzzone mit verstärkter Aktivität, 3 Unbeteiligte Seitenzone, 4 Epidermisblastem, 5 Corium.

Abb. 2. Mäuserückenhaut, quer, Gefrierschnitt, unfixiert. Vers. M 34, Bestrahlungszeit 30 min, Latenzzeit 196 Std., Nadi-Reaktion, Vergr. 35-fach. 1 Zentrum der bestrahlten Stelle, geringere Aktivität, 2 Regenerierende Grenzzone mit starker Aktivität, 3 Sehr weit seitlich liegendes Corium, leichte Reaktion, 4 Epidermis, 5 Corium.

(3) Regenerative Erscheinungen werden erst nach einiger Zeit sichtbar. Ein Beispiel für die erste Bedingung ist die Aktivierung des Peroxydase-Systems und der Tyrosinase. Beide Reaktionen treten bei genügend intensiver Bestrahlung an der Froschschwimmhaut bereits direkt nach der Bestrahlung auf. Bei der Mäusehaut dagegen zeigen sich die Reaktionen aber erst nach etwa 24 Std. Auch bei der Froschschwimmhaut wurde von geringeren Dosen nachgewiesen, dass direkt nach der Bestrahlung noch kein Effekt und nach etwa 24 Std. deutliche Aktivierung zu beobachten war. Hieraus kann geschlossen werden, dass in beiden Fällen eine reziproke Abhängigkeit der Zeit bis zum Reaktionsnachweis von der Dosis besteht. Der Grund für dieses Verhalten ist darin zu suchen, dass eine intakte Blutversorgung und eine hohe Sauer-

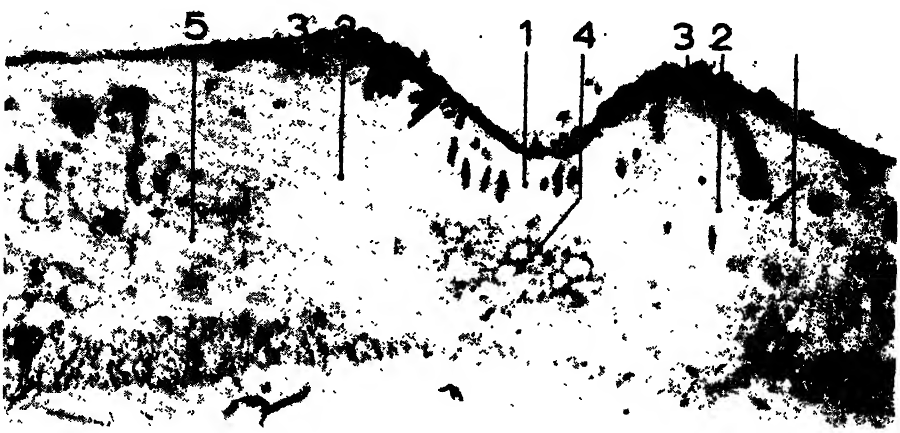

Abb. 3. Mäuserückenhaut, quer, Gefrierschnitt, unfixiert. Vers. M 34. Bestrahlungszeit 30 min, Latenzzeit 196 Std., Esterase-Reaktion, Vergr. 35-fach. 1 Zentrum der bestrahlten Stelle, vollständige Inaktivierung. 2 Regenerierende Grenzzone, Aktivität noch verringert, 3 Zone des Epidermisblastems und darunterliegendes Corium, Aktivität deutlich verstärkt, 4 Tiefer liegende Restaktivität im Zentrum der bestrahlten Stelle, 5 Seitenzone mit normaler Aktivität, entspricht der unbestrahlten Zone.

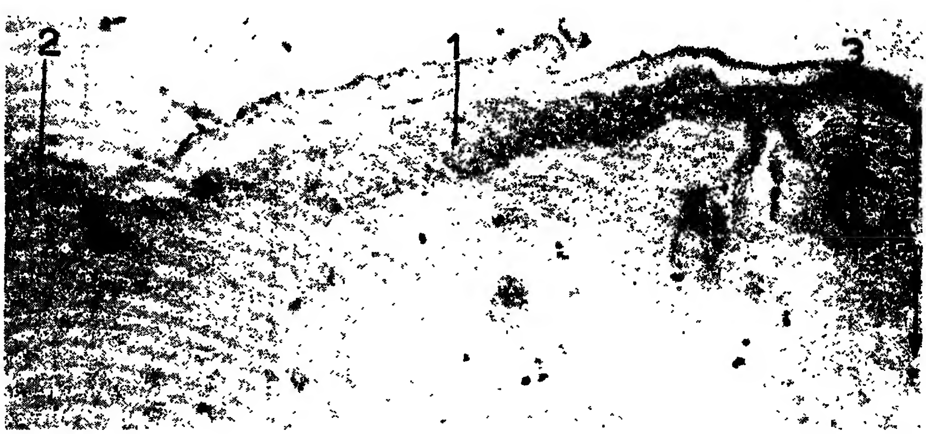

Abb. 4. Mäuserückenhaut, quer, Paraffinschnitt, fixiert mit Rossmann's Gemisch. Vers. M 23, Bestrahlungszeit 30 min, Latenzzeit 120 Std., RNS-Nachweis, Vergr. 60-fach. 1 Epidermisblastem in der Grenzzone, 2 Zentrum der bestrahlten Stelle, 3 Unbestrahlte Stelle, 4 Corium.

Literatur S. 341

stoffspannung im Gewebe Voraussetzung für die Reaktion sind. Werden abgeschnittene Häute bestrahlt oder intakt bestrahlte Häute längere Zeit bis zur Untersuchung abgeschnitten gehalten, dann tritt der Effekt nicht ein.

Unter die zweite Bedingung fällt die Inaktivierung der Dehydrogenasen, der alkalischen Phosphatase und der unspezifischen Esterase. Für diese Fermentinaktivierungen wurde nachgewiesen, dass auch sie bereits mit geringeren Dosen erzielbar sind, wenn bis zum Nachweis des Effektes eine Latenzzeit verstreichen kann. Gleichzeitig konnte aber deutlich gemacht werden, dass diese Effekte auch eintreten, wenn abgeschnittene Häute bestrahlt werden, bzw. die Häute nach Bestrahlung abgeschnitten belassen werden. Hierzu ist also die Zufuhr bestimmter Stoffe durch die Blutversor-

Abb. 5. Mäuserückenhaut wie Abb. 4, PAS-Reaktion auf Glykogen, Vergr. 60-fach. Bezeichung wie Abb. 4.

gung nicht nötig. Man kann diese Reaktionen also mit grosser Wahrscheinlichkeit als Primärreaktionen betrachten. Auch die Veränderungen der RNS-Moleküle gehören zu diesem Punkt. Hier lässt sich die Anfangsreaktion durch die erhöhte Basophilie besonders gut erkennen, der dann der Abbau folgt.

Zur dritten Erklärung für die notwendige Latenzzeit lässt sich eine Reihe von Aktivitätssteigerungen anführen. Bei der Mäusehaut vor allem, erhöhen sich um das bestrahlte Gebiet herum in einer Grenzzone, die grösstenteils zur bestrahlten Zone zählt, in geringerem Umfang auch unbestrahlte Regionen mitumfasst, nach mindestens 48 Std. die verschiedenen Fermentaktivitäten, die oben erwähnt wurden. Sowohl im Corium als auch in der Epidermis wird Steigerung der Aktivität der DPN Diaphorase und der alkalischen Phosphatase deutlich. Die unspezifischen Esterasen verstärken höchstens geringfügig ihre Aktivität. Die Oxydationswirkung dieser Grenzzone ist sehr hoch. Dies zeigt, dass hier Abbauprozesse ablaufen, die hohe Energiemengen benötigen und besondere Anforderungen an die Fermente des Differenzierungsvorganges stellen. In dieser Region ist auch sowohl in der Epidermis als auch im Corium der Gehalt an RNS bedeutend erhöht. Dies ist verständlich, da in dieser Region die Eiweisssynthese, in die die cytoplasmatische RNS eingeschaltet ist, in

hohem Masse verstärkt ist. Die Reaktion der RNS und der verschiedenen Fermente liegt im Grossen gesehen etwa an der gleichen Stelle. Sie beginnt nach etwa 50–60 Std. und neigt sich etwa 300 Std. nach der Bestrahlung dem Ende zu. Dies ist aber weitgehend abhängig von der Grösse des bestrahlten Areals. Im Corium läuft dieser Prozess parallel mit der Zuwanderung der verschiedensten Zellformen, die zum Aufbau der Coriumfasern in diesem Bereich führt. Das verdickte Epidermisblastem schiebt sich von beiden Seiten nach der Mitte zusammen.

Sehr interessant ist das Verhalten des Glykogens in diesem Blastem. Mittels der PAS-Reaktion und Kontrolle mit Diastase-Behandlung lässt sich nachweisen, dass in bestimmten Zellen des Epidermisblastems eine Anreicherung von Glykogen eintritt. Es sind Zellen, die im Blastem der bestrahlten Stelle zugerichtet sind. Die Bedeutung dieser Erscheinung ist weitgehend unklar. Versuche zur Klärung sind im Gange. Dieses Glykogen tritt ebenfalls nach etwa 50–60 Std. in Erscheinung und verschwindet aber schon nach 200 Std. wieder.

Die Abb. 1-3 zeigen diese Verhältnisse. Im bestrahlten Bereich sind nach einer Latenzzeit von etwa 100–200 Std. deutlich die Inaktivierung der DPN-Diaphorase, der Esterase, der alkalischen Phosphatase und der Abbau der RNS zu erkennen. In der Grenzzone und in der verdickten Epidermis zeigt sich verstärkte Aktivität. Die Oxydationswirkung ist besonders stark in der Grenzzone, aber auch im Zentrum noch erhöht. Die Abb. 4 und 5 geben die Verteilung von RNS und Glykogen im Epidermisblastem wieder.

LITERATUR

1 H. Bücker und W. Hanke, *Z. Naturforsch.*, 12b (1957) 629.
2 W. Hanke, *Z. Naturforsch.*, 14b (1959) 592.
3 W. Hanke, *Z. Zellforsch. u. mikroskop. Anat.*, 50 (1959) 644.
4 W. Hanke, *Enzymatische Veränderungen in der Haut nach Bestrahlung mit U.V.*, Vortrag gehalten auf der 1. Tagung der Deutschen Lichtforschenden Gesellschaft, Bad Nauheim, 1959.
5 P. Dubouloz und J. Dumas, in *Proc. of the 1st Intern. Congress on Photobiology, Amsterdam*, 1954, p. 247.
6 W. Hanke, *Z. Naturforsch.*, 12b (1957) 299.

LOWERING OF THE TEMPERATURE OF HUMAN SKIN AFTER IRRADIATION WITH ULTRAVIOLET

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Ultraviolet irradiation of human skin can give rise to changes in skin-temperature. It is well-known that severe sunburn erythemas may be warm. It is less well-known but occasionally reported in literature that ultraviolet-erythemas can also be "cool", in comparison with surrounding non-irradiated skin. We tried to measure these temperature-differences because we hoped in this way to find a feasible quantitative measure for the irradiation effects.

The measurements are carried out in a climate-room with regulated air temperature and humidity and the infrared radiation of the skin is detected by means of a thermopile. In this way it is possible to measure the skin temperatures continuously without appreciably disturbing the temperature-pattern.

As yet, we have only performed preliminary measurements. On the inner side of the fore-arm, erythemas were elicited with a diameter of 2–3 cm. These erythemas were mostly of the 300 mμ type. The temperatures were measured in the centre of the irradiated area and in two fixed positions a few cm outside it. As a measure of the temperature-effects of the irradiation we used $\triangle T$, the difference between the temperature at the centre of the irradiated area and the mean of the temperatures of the two other positions, diminished by the value of this same quantity before the irradiation.

The temperature-effects turn out to be quite variable and strongly dependent on conditions. It seems improbable that the temperature will be useful as a measure of the irradiation effects. However, some interesting points can be noted. The present discussion will be confined to what is measured during the interval between irradiation and the start of visible reddening of the skin, *i.e.* the delay-time of the erythema.

During this interval, the irradiated area is nearly always cool in comparison with the surrounding skin. This seems to be true in cases ranging from irradiation with sub-erythemal dose to those with rather severe doses, which later on give rise to warm erythemas. The effect is small, $\triangle T$ being usually not more than -0.1 to $-0.2°$. We are interested in it, however, because we know hardly any established fact in relation to what is happening in the skin during the delay-time.

When does this cooling begin? At the moment this question cannot be answered with certainty. Immediately after the irradiation the temperature-pattern of the skin is disturbed. The irradiated area is heated by the radiant energy, the surrounding skin

by the screen which has covered it during the irradiation. Thus it is necessary to wait until a stationary state is established. After about 0.5 h the effect is nearly always present. This is long before the start of the reddening with the doses used.

What is the cause of the decrease in temperature? To answer this question definitively more measurements will be needed in order to see how the effect depends on conditions. However, there are some indications that it is caused by increased evaporation of water from the skin.

For existing erythemas Felsher and Rothman[1] demonstrated by means of direct evaporation measurements that the normal insensible perspiration was increased by 20 to 40%. This was measured 24 h after irradiation.

We measured ΔT 10 h after an irradiation and then suddenly raised the relative humidity of the air in the room from 25 to 90%. ΔT, which was about $-0.2°$ before this operation, nearly disappeared. It returned to about the original value when the humidity was lowered again.

It seems that in this case there was increased evaporation from the erythema 10 h after irradiation. We have not made such measurements sooner after irradiation but the continuity of the curve of ΔT *versus* time suggests that the same effect already existed less than one hour after irradiation. An estimation of the amount of excess evaporation needed for such a temperature decrease can be made by a rough calculation.

During a certain time interval an area of skin dissipates a quantity of heat H, partly by radiation and conduction, partly by evaporation. Supposing that in a case without sensible sweating the insensible perspiration accounts for 10% of the heat dissipation, or 0.1 H, the remaining 0.9 H is left for radiation and conduction. The latter dissipation is roughly proportional to the difference between skin temperature and environment temperature. Supposing this difference to be about 10°, a temperature decrease of 0.1° diminishes the heat dissipated by these processes by 1% or roughly by 0.01 H. In order to increase the dissipation by evaporation by this same amount, the evaporation must be increased by 10%. Thus, the measured ΔT of -0.1 to $-0.2°$ could be accounted for by an increase of insensible perspiration from 10 to 20%. This would agree fairly well with the increase of 20 to 40% found directly by Felsher and Rothman for more developed and probably more severe erythemas.

There is another indication of increased evaporation from the irradiated skin area. It is generally believed that during the delay-time nothing can be seen on the skin. However, especially after irradiation with 250 mμ radiation, a slight darkening of the skin is often perceptible from about 1 h after irradiation even in cases in which the reddening only occurs after 5 h or more. This darkening of the skin without any change in colour is best observed in soft, diffuse light in a half dark room.

It is attractive to think that what we see here is another facet of the same fact: a decrease of light scattering in the stratum corneum by imbibition of this layer with fluid from which water is evaporating at the surface. The decrease of scattering fractionally diminishes the white light returning from the skin thus giving the darkening.

As a test of this hypothesis we tried to imitate the darkening by applying water to the skin. An area of skin was wetted with a soft brush over 0.25 h. When this had been done — on a fair, dry skin — a darkening which was quite similar in appearance appeared. This "water-darkening" is also best observed in the half dark. This observation is thought to support the hypothesis that increased evaporation occurs quite quickly after irradiation. In any case there is an effect here, possibly on the vessels of

Reference p. 344

the skin, that occurs long before the widening of the vessels which is seen as reddening of the skin.

After many years of research and theory the processes occurring during the delay-time of the erythema are still unknown. More observations seem to be required on these processes some of which must be essential for the understanding of the erythema of sunburn. It is hoped that the early effect discussed here will be of some help in this direction.

REFERENCE

1 Z. Felsher and S. Rothman, *J. Invest. Dermatol.*, 6 (1945) 271.

UNSERE UNTERSUCHUNGEN ÜBER DIE WIRKUNGSWEISE DES ULTRAVIOLETTEN LICHTES BEIM GESUNDEN ERWACHSENEN

U.V.-BESTRAHLUNG UND VITAMIN C-SPIEGEL

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In zahlreichen Untersuchungen konnte gezeigt werden, dass durch *erythemawirksame* U.V.-Bestrahlungen und nur durch diese die Leistungsfähigkeit des gesunden Erwachsenen erhöht werden kann. Der in unserem Sinne mit Erfolg bestrahlte Mensch — es sind nicht alle — kann bei gleicher Kreislaufbelastung eine höhere Leistung vollbringen. Die nach Abklingen des Erythems auftretende Wirkung der Ultraviolettbestrahlung deckt sich nicht mit der antirachitischen U.V.-Wirkung.

Indem wir versuchten der Wirkungsweise der U.V.-Bestrahlung näherzukommen, sahen wir in den Untersuchungen der letzten Jahre eine Reihe von Wirkungen, die auf eine engere Verknüpfung der U.V.-Wirkung mit dem Vitamin C-Stoffwechsel hinzudeuten schienen. Ich nenne in rascher Folge einige dieser Befunde.

Die Ausscheidung der 17/21 Hydroxy-20-ketosteroide steigt bei einigen, aber nicht bei allen Versuchspersonen unter der zu einer Lichtentzündung der Haut führenden U.V.-Bestrahlung an (Abb. 1).

Setzt man voraus, dass Nebennierenrinden-Aktivität und Nebennierenrinden-

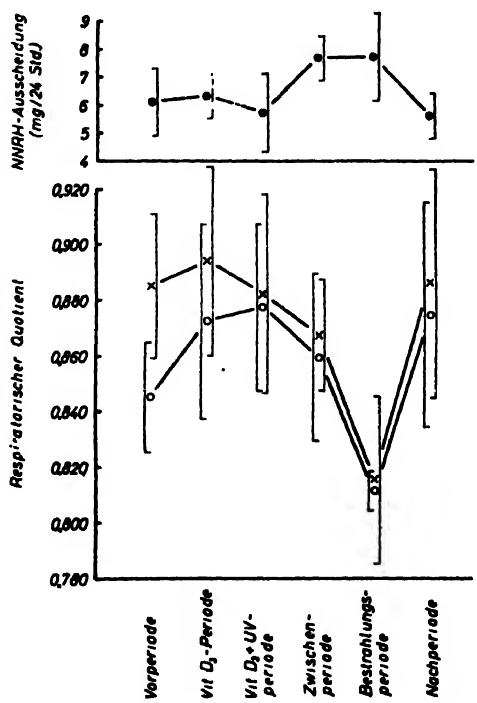


Abb. 1. 17,21-Dihydroxy-20-ketosteroid-Ausscheidung und respiratorischer Quotient nach U.V.-, Vit. D$_3$-, U.V. + Vit. D$_3$-Behandlung. Mit dreifachem mittleren Fehler des Mittelwertes: ● NNRH-Ausscheidung; × RQ im Arbeitsumsatz; o RQ im Grundumsatz.

Literatur S. 349

Ausscheidung parallel gehen, so wird durch die U.V.-Bestrahlung die Nebennierenrinde aktiviert. Die engen Beziehungen zwischen Nebennierenrindenaktivität und dem Absinken des Vitamin C-Spiegels der Nebenniere sind geläufig. Die Hormone der Nebennierenrinde sind eng verknüpft mit dem Muskelstoffwechsel und der Muskelkraft.

Skeletmuskulatur, die man täglich einige Male isometrisch kontrahiert, wird trainiert. Zusammen mit Hettinger[2] konnten wir zeigen (Abb. 2), dass die Kraftzunahme

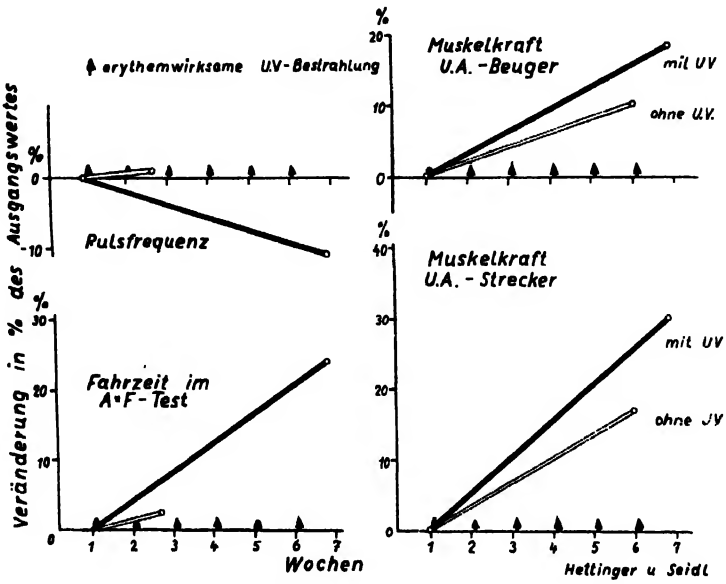


Abb. 2. U.V.-Wirkung und Muskeltrainierbarkeit

durch den gleichen Trainingsreiz grösser ist, wenn der Trainierende einmal wöchentlich erythemwirksam bestrahlt wird. Sokolova[5] nimmt an, dass die für den Muskelstoffwechsel wesentliche Adenosintriphosphatase durch Vitamin C geschützt wird.

U.V.-Bestrahlung des Menschen beeinflusst aber auch den Kohlenhydratstoffwechsel (Abb. 3). So liegt der ATP-Spiegel des Blutes während einer Serie von U.V.-Bestrahlungen niedriger als vorher und nachher[3].

Und schliesslich sei erwähnt, dass die Ascorbinsäure in den Abbau der aromatischen Aminosäuren, insbesondere des Tyrosins eingreift. Diesem kommt aber als wichtiger Ausgangssubstanz der Melaninbildung bei der dem U.V.-Erythem folgenden Pigmentierung besondere Bedeutung zu.

Die Vielzahl der Verknüpfungen von Vitamin C mit den von uns beobachteten Folgen der U.V.-Bestrahlung machten es daher wünschenswert, den Vitamin C-Spiegel des Serums nach U.V.-Bestrahlung bei gesunden Erwachsenen zu beobachten. Die ersten Befunde der in Zusammenarbeit mit Frau Dr. Starlinger durchgeführten Untersuchungen seien kurz mitgeteilt. Als Bestimmungsmethode des Vitamins C wurde die von Roe und Kuether[4] angewandt.

Wir untersuchten den Vitamin C-Spiegel im Serum in mehreren verschiedenen Versuchsreihen. Bei der ersten Versuchsreihe wurden 2 ♀ Versuchspersonen in 8 aufeinander folgenden Wochen einmal wöchentlich bestrahlt. Die Bestrahlung erfolgte als Kurzzeitbestrahlung, d.h. die Erytheme wurden mit wenigen Sekunden Bestrahlungszeit durch das Hanauer Kurzzeitbestrahlungsgerät erzielt. Die Blutent-

nahmen aus der Fingerbeere wurden bei den nüchternen Versuchspersonen 2 Stunden vor der Bestrahlung, 24 Stunden nach der Bestrahlung im Erythemstadium und 3

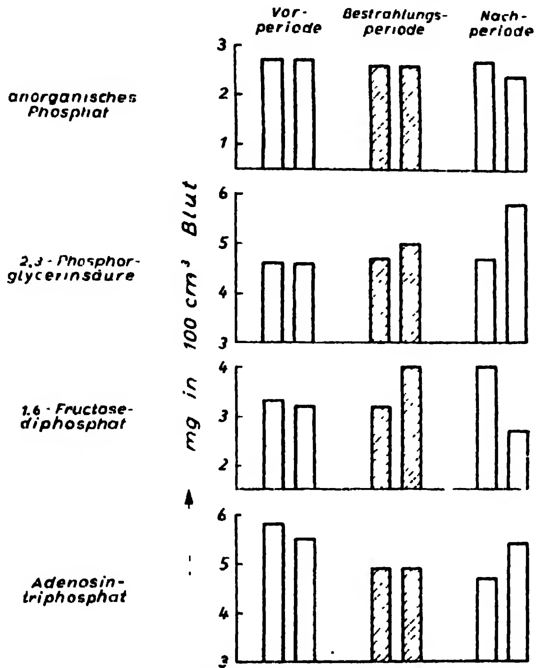


Abb. 3. Die Fraktionen des säurelöslichen Phosphates im Blut nach U.V.-Bestrahlung

Tage nach der Bestrahlung, wenn die Erytheme bereits abgeklungen waren und sich die Pigmentierung deutlich hervorhob, vorgenommen.

Es zeigt sich, dass der Ascorbinsäurespiegel im Serum nach Bestrahlung im Erythemstadium niedriger liegt als vorher und signifikant niedriger ist als im Pigmentstadium (Tabelle I). In der Nachperiode wurden die Blutentnahmen an den entsprechenden Tagen vorgenommen. Der Ascorbinsäurespiegel zeigte jedoch nicht den in der Bestrahlungsperiode beobachteten Gang.

In einer zweiten Serie wurde die U.V.-Bestrahlung *täglich* bei gesunden 5 Versuchspersonen gegeben, und zwar ähnlich dem Schema von Rollier bei den Füssen beginnend aufsteigend.

Ich zeige als Beispiel das Verhalten eines 29-jährigen Mannes (Tabelle II). Als Bestrahlungsquelle diente der normale Hanauer S 500 Brenner. Alle Bestrahlungen zeitigten ein Erythem. Die Versuchsreihe dauerte nach einer Vorperiode von 3 Wochen im ganzen 2 Monate. Es wurden innerhalb von 14 Tagen 11 Bestrahlungen gegeben. Die nächste Abbildung (Abb. 4) zeigt einige Ergebnisse des Versuches. Auf der Abszisse sind die Werte von je 2 Versuchstagen gemittelt.

Betrachten wir zuerst den Test von Lehmann-Michaelis, der uns Aufschluss über die bei gleicher Kreislaufbelastung mögliche Leistung gibt. Die in der Vorperiode recht gleichmässige Fahrzeit steigt nach den ersten Bestrahlungen deutlich an, lässt aber dann sehr bald wieder nach, unter dem Einfluss der stets erythemerzeugenden Bestrahlung um auch nach Absetzen der Bestrahlung in der knapp 14-tägigen Nachperiode nicht wieder anzusteigen.

Literatur S. 349

TABELLE I

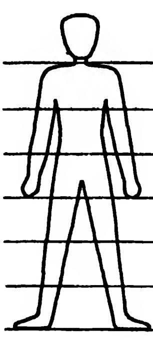
RANGFOLGE DES ASCORBINSÄUREGEHALTES IM SERUM BEI VERGLEICH VON JE 3 BESTIMMUNGEN PRO WOCHE

Woche		*Vp. Ru* 2 Std. vor Bestr.	24 Std. nach Bestr.	72 Std. nach Bestr.		*Vp. Lü* 2 Std. vor Bestr.	24 Std. nach Bestr.	72 Std. nach Bestr.
UV-Bestrahlungsperiode								
B_1		1	3	2		1	3	2
B_2	E	2	1	3		1	2	3
B_3		2	2	1	E	2	1	3
B_4	E	2	1	3	E	1	2	3
B_5	E	2	1	3	E	1	2	3
B_6		1	2	3	E	3	1	2
B_7	E	1	1	2		1	2	3
B_8	E	2	1	1	E	2	1	3
B_9	E	3	1	2			.	...
Mittelwert B_1-B_9		1.8	1.4	2.2		1.5	1.8	2.8
Mittelwert 6	E	2.0	1.0	2.3		1.8	1.4	2.8
Mittelwert 3		1.3	2.3	2.0		1.0	2.3	2.7
Nachperiode								
NP_1		.				---	—	-
NP_2		1	2	1		1	2	1
NP_3		2	1	3		1	2	1
NP_4		3	2	1		1	2	3
NP_5		3	2	1		3	2	1
MW NP_{1-5}		2.3	1.8	1.5		1.5	2.0	1.5

E = Erythem

TABELLE II

Bestrahlungsschema für Vp. Schö. (liegend)



Zone	Bestrahlungszeit in Minuten für die einzelnen Zonen bei Bestrahlung. 1 (23.9.)	2 (24.9.)	3 (25.9.)	4 (26.9.)	5 (28.9.)	6 (29.9.)	7 (30.9.)	8 (1.10.)	9 (2.10.)	10 (3.10.)	11 (5.10.)
6						2	x	x			
5					3	5 E	3				
4				[illegible]	6	8 E	6	3			
3			3	5 E	5 E	12 E	9 E	6	3		
2		4	6 E	8 E	9 E	12 E	12 E	9 E	6	3	
1	4 E	8 E	9 E	11 E	12 E	12 E	12 E	9 E	6 E	3	3

E = Erythem
x = Herz- und Magenbeschwerden

Verwendete UV-Quelle: Hanauer Quarzlampe S 500
Abstand Lampe-Versuchsperson: 1 m

Vergleichen wir nun damit das Verhalten der Ascorbinsäure, so fällt sie unter der Bestrahlung signifikant um etwa 30% ab. Die Versuchsperson war während des Versuches durch besonders reichlichen und gleichmässigen Obstgenuss mehr als ausreichend mit Vitamin C versorgt. Als der Vit. C-Spiegel im Serum auf Werte unter 0.9 mg% absank, begann die Versuchsperson über Herz- und Magenbeschwerden zu klagen, U.V. war überdosiert.

Der Sauerstoffverbrauch lag während der gesamten Bestrahlungsperiode höher als

n der Vorperiode. Es steht diese Tatsache nicht im Gegensatz zu unseren sonstigen Befunden, da wir die Senkung des Grundumsatzes erst nach dem Abklingen des akuten Erythems fanden. Der eben geschilderte Versuch wurde noch mit einer 24-

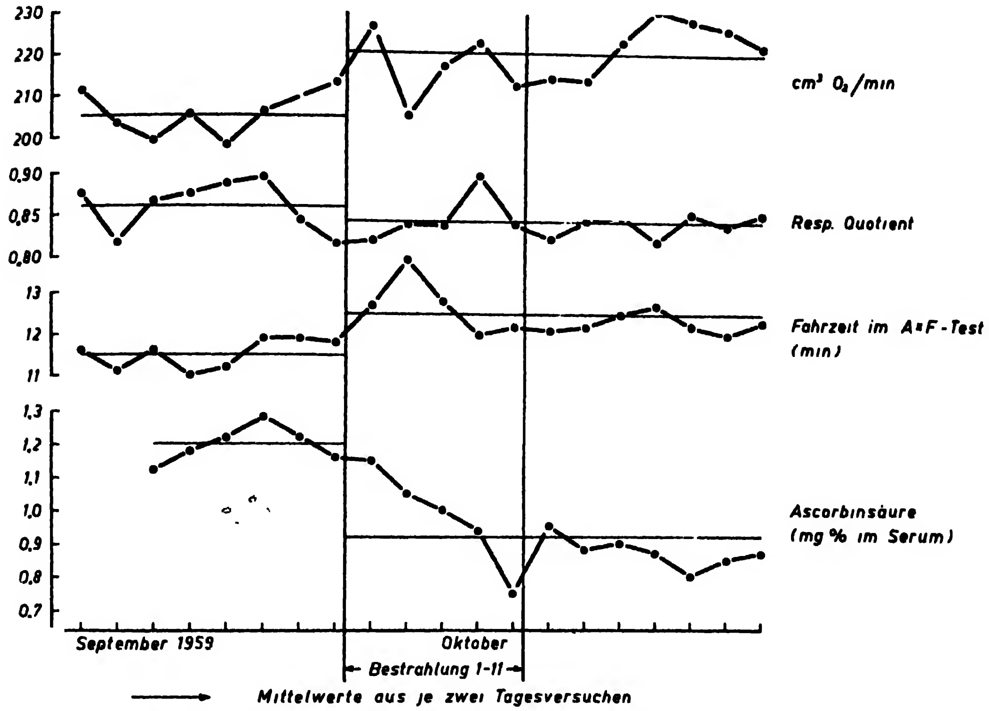


Abb. 4. Sauerstoffverbrauch, Resp. Quotient, Fahrzeit im A + F-Test und Ascorbinsäurespiegel im Blut unter dem Einfluss täglicher U.V.-Bestrahlungen (Vp. Schö).

jährigen ♂ Versuchsperson wiederholt. Hier trat das Erythem erst nach der 15. Bestrahlung auf. Zugleich senkte sich der Ascorbinsäurespiegel signifikant um 10%. Es ergibt sich demnach wie ich an 2 Beispielen zu zeigen versuchte dass unter dem Einfluss einer erythemwirksamen U.V.-Bestrahlung ein Absinken des Ascorbinsäurespiegels im Serum während des akuten Erythems festzustellen ist, dem möglicherweise im Pigmentstadium eine Erhöhung folgt.

Aus den Versuchen lässt sich entnehmen, dass ähnlich wie bei den von Douzou und LeClerc[1] *in vitro* gemachten Befunden auch *in vivo* durch U.V.-Bestrahlung ein erhöhter Verbrauch von Ascorbinsäure festzustellen ist. Vitamin C verhält sich wie ein "Substrat", indem es verbraucht wird.

Die Entscheidung, ob wir es mit einer unspezifischen Reaktion auf den "Stress" der Besfrahlung oder einer spezifischen U.V.-Reaktion zu tun haben, bleibt weiteren Untersuchungen vorbehalten. Doch neigen wir zu der Erklärung, dass der durch die Bestrahlung gesetzte Stress Ursache des erhöhten Ascorbinsäureverbrauchs ist.

LITERATUR

1 P. Douzou und A. M. LeClerc, *Compt. rend.*, 244 (1957) 3093.
2 Th. Hettinger und E. Seidl, *Intern. Z. angew. Physiol.*, 16 (1956) 177.
3 E. Seidl, *Strahlentherapie*, 111 (1960) 359.
4 I. H. Roe und C. A. Kuether, *J. Biol. Chem.*, 147 (1943) 399.
5 V. E. Sokolova, *Biokhimiya*, 21 (1956) 465.

DIE WIRKUNG KURZDAUERNDER INTENSIVER U.V.-BESTRAHLUNG

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Ein erhebliches Hindernis für die Durchführung von Ultraviolettbestrahlungen an grösseren Menschengruppen bildet der Zeitaufwand, der bei Verwendung der gebräuchlichen Hochdruck-Quecksilberlampen erforderlich ist, zumal die Bestrahlungsdauer von einer Bestrahlung bis zur anderen um etwa 25–30% gesteigert werden muss. Aus diesem Grunde veranlassten wir die Quarzlampengesellschaft in Hanau, uns eine Lampe herzustellen, die über ein Zeitrelais kurzzeitig wesentlich höher belastet werden kann. Mit Hilfe eines solchen "Blitzes" stieg die Intensität der Strahlung, die beim Dauerstrom etwa einem Brenner S 500 gleichkam, auf ungefähr das 13-fache an. Es musste untersucht werden, ob die biologische Wirkung auch bei verkürzter Bestrahlungszeit und entsprechend erhöhter Strahlenintensität vorhanden ist.

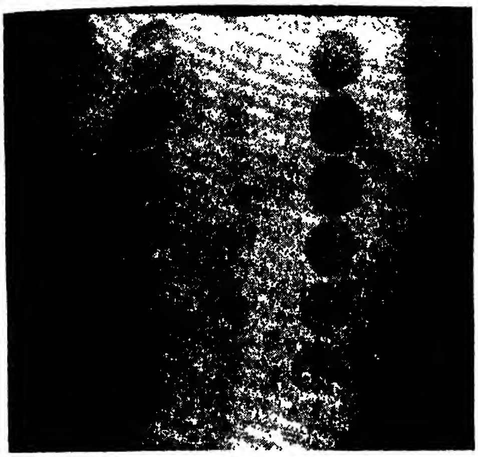
Hierbei zeigte sich, dass die bekannte Kette der Bestrahlungsfolgen auch bei der intensiven Blitzbestrahlung von wenigen Sekunden Dauer vorhanden war. Es konnte ein Erythem hervorgerufen werden, auf dessen Abheilung Pigmentbildung folgte. Es waren auch die typischen Veränderungen des Stoffwechsels vorhanden, die zur Steigerung der Leistungsfähigkeit führen.

Im einzelnen aber zeigten sich nicht unerhebliche Unterschiede. Das Erythem trat schon nach 1 bis 2 Stunden, also schneller auf und war ausgesprochen kleinfleckig. Es zeigt in etwa das Bild, das man bei Bestrahlung mit der Wellenlänge von 254 mμ zu sehen gewöhnt ist. Hiermit stimmt überein, dass das Spektrum der Lampe eine geringe Verschiebung der Energieverteilung in Richtung auf die kürzeren Wellenlängen zu zeigen scheint. Ein weiterer Unterschied lag darin, dass nach Verschwinden des Erythems eine ganz besonders intensive und langanhaltende Pigmentierung auftritt.

Abb. 1 zeigt rechts eine Punktreihe im Erythemstadium, die mit Blitzen von 8.7; 6.1; 4.3; 3.1; 2.3; 1.6; 1.1 Sekunden Dauer gewonnen ist. Die linke Punktreihe ist mit einem S 500-Brenner und jeweils der 20-fachen Bestrahlungsdauer erzeugt. Im Erythemstadium ist kein wesentlicher Unterschied zwischen den beiden Reihen erkennbar.

40 Tage später aber ist, wie Abb. 2 zeigt, von der mit dem normalen Brenner erzeugten Punktreihe so gut wie nichts mehr zu sehen, während die rechte Punktreihe nunmehr mit schön ausgebildeten Pigmentflächen noch vollkommen deutlich vorhanden ist. Die Ursache dieser verstärkten Pigmentierung vermögen wir noch nicht zu erklären.

In einer früheren Untersuchung hatte Seidl gezeigt, dass bei oligochromatischer Bestrahlung je nach den verwendeten Wellenlängen auch Unterschiede in dem Modus der Stoffwechselwirkung erkennbar sind. Im mittleren Feld des nächsten Bildes (Abb. 3) ist bei ein und derselben Versuchsperson die Beeinflussung der Leistungsfähigkeit



bb. 1. Teststreifen zur [illegible] emschwelle. 24 Std. nach Bestrahlung. nks: Hanauer Quarzlampe S 500; rechts: UV-Blitzgerät. Vp.: P.S.

Abb. 2. Pigmentierung der Teststellen. 40 Tage nach Bestrahlung. links: Hanauer Quarzlampe S 500; rechts: UV-Blitzgerät. Vp.: P.S.

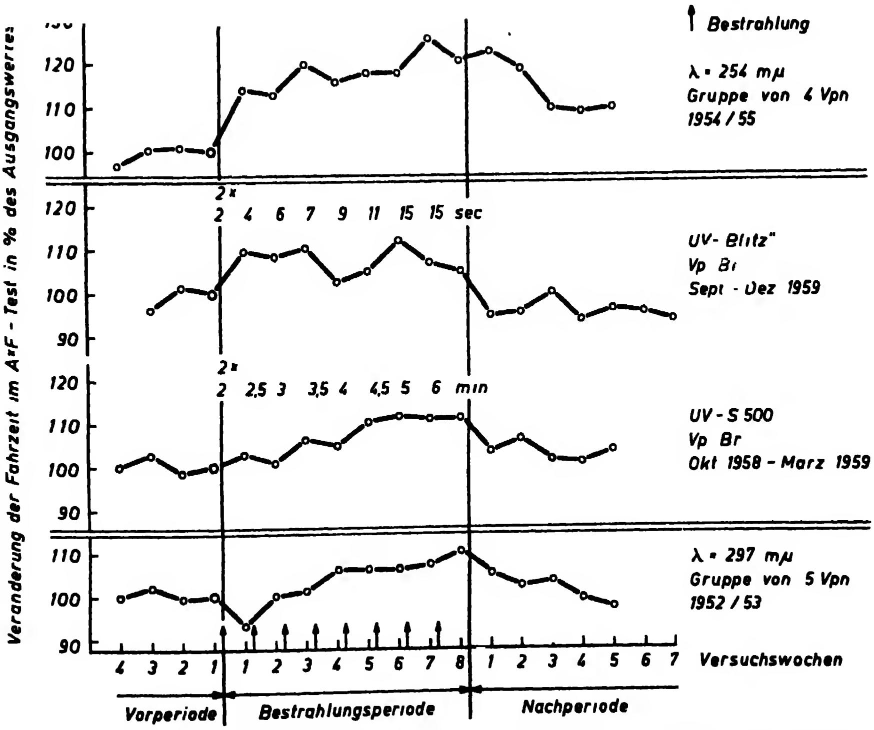


Abb. 3. Beeinflussung der Leistungsfähigkeit einer Versuchsperson (♂) durch 8 U.V.-"Blitze" bzw. 8 QL-S 500 – Bestrahlungen.

durch die Quarzlampe S 500 der Beeinflussung durch eine Serie von "Blitzen" gegenübergestellt. Die Versuche wurden im Abstand von 9 Monaten durchgeführt.

Bei Behandlung mit der Quarzlampe ähnelt der stete Anstieg der Leistungsfähigkeit dem Verhalten einer Versuchspersonengruppe, die oligochromatisch mit $\lambda = 297$ mμ bestrahlt worden war. Der steile Anstieg der Fahrzeit nach "Blitzbehandlung" kommt dem durch Bestrahlung mit $\lambda = 254$ mμ erzielten Effekt näher. Wenn auch unser Material nicht ausreicht, um das Typische dieser Verlaufsformen zu sichern, so spricht dieser Befund doch für die auf Grund der Erythembeobachtung gewonnene Vorstellung, dass eine Verschiebung der Wellenlängen nach der kurzen Seite eine Rolle spielt.

Ein weiterer Unterschied der Strahlenwirkung des U.V.-"Blitz"-Gerätes gegenüber den normalen Hochdrucklampen besteht ferner darin, dass eine viel geringere prozentuale Verlängerung der Bestrahlungszeiten erforderlich wird. Bei wöchentlich einmaliger Bestrahlung braucht in der Regel nach der 3. oder 4. Bestrahlung nur eine Verlängerung um 10% der Bestrahlungszeit durchgeführt zu werden, um die gleiche Erythemwirkung zu erzielen. Es sieht also so aus, als ob die Verdickung der Hornhaut, die Bildung der dauerhaften Lichtschwiele, bei der Anwendung der Blitzlampe in geringerem Masse eintritt.

Ich fasse zusammen: Die kurzzeitige intensive Bestrahlung (mit einem neuen U.V.-"Blitz"-gerät) verursacht einen Erythemcharakter, wie er bei kurzwelligem U.V. beobachtet wird. Ebenso verhält sich die Stoffwechselwirkung. Die Pigmentbildung ist nicht flüchtig wie bei kurzwelliger Bestrahlung, sondern ähnelt in Stärke und Dauer derjenigen des langwelligen U.V.. Die Verdickung der Hornhautschicht geht langsamer vor sich. Wir müssen uns mit der Mitteilung dieser Befunde begnügen, ohne heute schon eine Erklärung wagen zu können.

THE RADIATION AREA OF THE ERECT HUMAN BODY WITH RESPECT TO THE SUN

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One of the factors affecting man's performance outdoors is the energy he receives from the sun in the form of radiation. It is of interest to the physiologist to study the influence of radiant energy on the body, but in order to do so he must have some idea of the magnitudes of the energies involved.

Radiant energy reaches a man exposed outdoors by several routes, directly from the sun, by reflection from the surrounding terrain, by scattering from the sky, and by re-radiation from hot surroundings.

The amount of energy incident upon the body directly from the sun may be estimated, if the normal solar intensity is measured, and the area of the body projected normally to the solar rays is known, the product yielding the required quantity. Clearly, the solar altitude, the body posture, and its orientation with respect to the sun, influence the result.

METHODS

In order to find values for the normal projected areas (Direct Radiation Area, D.R.A.) a photographic method has been devised. An automatic camera and flash unit are mounted on a platform which may be moved in a quadrant of 4.67 m radius. The subject stands on a turntable below the centre of the quadrant. An operator makes exposures at known angles of altitude between 0° and 90°, the turntable is then rotated and the exposures repeated. The process continues until the subject is moved through an azimuth angle of 180°. In this manner a series of photographs are obtained which correspond to view-points situated on the surface of a hemisphere surrounding the subject.

The relevant projected areas are found from images of the negative, either by means of a planimeter, or with the aid of a photoelectric analyser. Since the radius of the quadrant is not large compared with the heights of the subjects, it is necessary to correct the results to correspond to an infinitely distant view-point.

RESULTS

Measurements so far have been confined to fifty subjects, both male and female, clad in the minimum of tightly fitting clothing and in the erect posture. The shapes of the projected areas, seen from the camera positions, are shown in Fig. 1 for two angles of azimuth. The forward facing direction has been arbitrarily selected as 0 deg. in the azimuth plane.

References p. 356

The surface areas of the subjects varied between 2.16 and 1.56 m² for males, and 1.73 and 1.59 m² for females. The surface areas were determined by the well known method of Dubois[1]. The D.R.A. changes only slightly with surface area and there is little difference between the sexes.

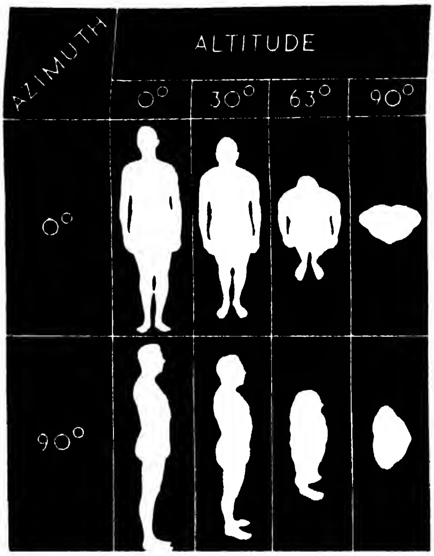

Fig. 1. Photographic silhouettes of erect male figures taken from points on a hemisphere surrounding the subject

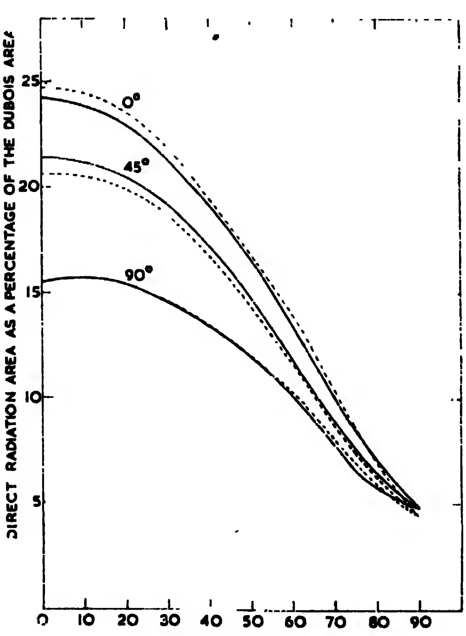

Fig. 2. Curves relating the area of nude erect male and female subjects projected normal to the suns rays with solar altitude for three angles of azimuth *i.e.*, 0, 45 and 90 degrees. ——— male; - - - - - female.

In Fig. 2 mean values of D.R.A. for all the subjects, expressed as percentages of ...ly surface area, are plotted against altitude for three azimuth angles. The curves ...ow the rapid decrease in D.R.A. with increasing altitude, when this is large.

The full line curve of Fig. 3 represents the mean D.R.A's taken without reference ...o the angle of azimuth for an average male subject. The graph may be considered as ...aving been derived from the curves of Fig. 2 by finding mean values for the ordinates ...n terms of true area.

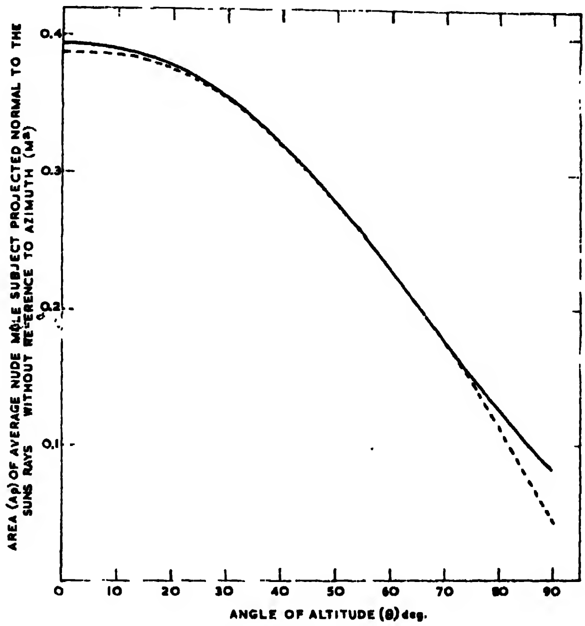

Fig. 3. Direct radiation area of an erect body compared with that of a vertical cylinder. - - - - - - - calculated values; —— observed values. Values calculated from the eqn $Ap = 0.0429 \sin \theta + 0.3845 \cos \theta$, which is the equation to the area of a right circular cylinder of height 1.649 m and radius 0.117 m in a direction normal to the sun rays. R.M.S. deviation between calculated and observed values = $\pm$ 0.0158 m².

It may be shown that the equation:

$$Ap = A \cos\theta + B \sin\theta \qquad (1)$$

represents the area (Ap) of a right circular cylinder projected towards an infinitely distant point at angular altitude θ, the axis of the cylinder being vertical. In equation (1)

$$A = 2\,rh,\ B = \pi\, r^2$$

where h and r, are the height and radius respectively. The broken line curve of Fig. 3 which lies close to the full line curve has equation:

$$Ap = 0.3845 \cos\theta + 0.0429 \sin\theta$$

whence,

$$A = 0.3845, \quad B = 0.0429.$$

References p. 356

Hence the cylinder most closely corresponding to the observated data has a radius of 11.7 cm and a height of 1.649 m.

The representation of the D.R.A. of the erect body by that of a cylinder is only justified if the exposure to the sun occupies equal periods of time with respect to all angles within any quadrant of the azimuth plane. This is the consequence of taking mean values for the ordinates. Present indications are that a fair representation of the D.R.A., having regard to azimuth angles, would be possible in terms of a right elliptical cylinder, but further analysis is required.

CONCLUSIONS

The necessarily brief description of the results which has been given above, serves to illustrate that the method is capable of providing some guidance concerning the direct component of the solar heat load on man; it should be emphasised, however, that the results are of a somewhat tentative nature and that much remains to be learned. The much wider problem of the absorption of radiant heat by the body outdoors, taking into account the many other factors, is being studied.

ACKNOWLEDGEMENTS

The writers wish to acknowledge the helpful advice given by Dr. T. Bedford under whose direction the work was commenced, and the guidance and interest given by Dr. O.G. Edholm who now directs the work. The writers would also like to thank Mr. R.W.T. Geary for his valuable assistance during the construction of the apparatus and with much of the task of analysis.

REFERENCES

1 D. Dubois and E. F. Dubois, *A.M.A. Arch. Internal Med.*, 15 (1915) 868.
2 T. Bedford, *J. Hyg.*, 35 (1935) 303.
3 T. Bedford, *Proc. Nutrition Soc. Engl., and Scot.*, 17 (1958) 167.
4 H. F. Blum, *J. Clin. Invest.*, 24 (1945) 712.
5 H. Bodenkamp and H. W. Ernst, *Arch. ges. Physiol., Pflüger's*, 229 (1931) 40.
6 A. Guibert and C. L. Taylor, *J. Appl. Physiol.*, 5 (1952–53) 24.
7 J. D. Hardy and A. M. Stoll, *J. Appl. Physiol.*, 7 (1954–55) 200.
8 J. L. Threlkeld and R. C. Jordan, *Heating Piping Air Conditioning*, 29 (1957) 135.
9 H. M. Vernon, *Quart. J. Roy Meteorol. Soc.*, 59 (1933) 239.

Session 1

PLANT PHOTOBIOLOGY

Chairman: ONNI POHJAKALLIO, Viik, Malmi (Finland)

Secretary: OLE SCHARFF, Copenhagen (Denmark)

Light effects on active uptake of sulphate by isolated leaf pieces

Burström[1] pointed out that on the basis of the classical concept of salt respiration (see for instance Lundegårdh[2]) one should expect ion accumulation in green tissues to be inhibited by light, since the oxygen gradient ought to be more or less reversed during photosynthesis. Nevertheless the available evidence pointed almost without exception in the opposite direction, and experiments were undertaken to elucidate this discrepancy.

Almost all earlier investigators used aquatic species, so the present author began with *Vallisneria gigantea* but then turned successively to the moss *Thuidium tamariscinum*, the succulent *Crassula argentea*, and finally to wheat (*Eroica* variety) and to the mutant barley "Bonus X_1–49", which segregates into green and albina plants. Pieces of the green tissues were prepared and suspended in complete nutrient solutions containing 0.05 m*M* sulphate for *Vallisneria* and *Thuidium*, 0.5 m*M* for the others. After 1–4 days in light the experiments were performed using the same type of solutions with an addition of "carrier free" radiosulphate added as a tracer. The experimental conditions were light (9,000 Lux) or darkness; 25° and aeration were used both during pretreatments and experiments. The nature of the process under investigation as an accumulation against a concentration gradient was established, and errors due to passive uptake within the free space, changing permeabilities, or the incorporation of sulphate S into organic fractions were excluded by control experiments. During these it appeared that the last-mentioned process is dependent upon a special, light-sensitive uptake mechanism, different from the one leading to accumulation of inorganic sulphate, at least as regards the rate-limiting steps.

The experiments show that for all the species tested KCN and 2,4-dinitrophenol (DNP) inhibit sulphate accumulation more in darkness than on illumination, *Vallisneria*/DNP being the only definite exception. On the other hand selenate, which competes specifically with sulphate, has the same action in light as in darkness. These facts are interpreted as meaning that the delivery of energy for sulphate accumulation is due to different processes in light and in darkness.

It was further observed that in the present material light sometimes increases and sometimes decreases the accumulation. This must logically be explained as due to at least partial inhibition of the dark uptake mechanism upon illumination; the process dependent upon illumination can of course not function in darkness. In *Crassula* it was possible to correlate the action of light with the intensities received before the preparation of the material for an experiment, indicating that after a period of bright illumination the experimental light tends to increase the sulphate accumulation. This is reversed under darker conditions. The light-effects mentioned must operate *via* the chloroplast pigments, since they do not occur in wheat roots or albino barley leaves.

The above results in the first instance remove the discrepancy between theory and experiment which was noted by Burström[1]. Salt respiration may in fact be inhibited on illumination of green tissues although the results are more or less concealed by the

References p. 360

initiation of a light-dependent mechanism of salt absorption operating through cytochrome f, which is known to be oxidized in illuminated chloroplasts[3]. On the other hand, it is also possible to interpret the results in terms of an ion uptake mechanism dependent upon high energy phosphates. This was suggested *e.g.* by Robertson Wilkins and Weeks[4]. In darkness such a mechanism must depend upon respiration. When photosynthetic phosphorylation[5] comes into play it might well, however, draw upon a common pool of phosphate acceptors to such an extent that respiration is inhibited and the energy delivery in sulphate accumulation switched over to photosynthetically produced adenosine-triphosphate and similar substances; such a chain of events would evidently explain the present results.

The properties of the sulphate ion should allow it to receive energy by both the mechanisms suggested above, since salt respiration ought to work on all anions and "active sulphate" is in many cases known to correspond with adenosine-3'-phosphate 5'-phosphosulphate[6]. As a matter of fact, further experiments with different inhibitors have given data which fit well with a phosphorylative mechanism, but some effects not easily interpreted in such terms have also been observed and both systems may possibly be at work at the same time.

The findings presented here are extensively published elsewhere[7-9].

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1 H. BURSTRÖM, *Fortschr. Botan.*, 19 (1957) 221.
2 H. LUNDEGÅRDH, *Symp. Soc. Exptl. Biol.*, 8 (1954) 262.
3 H. LUNDEGÅRDH, *Physiol. Plantarum*, 7 (1954) 375.
4 R. N. ROBERTSON, M. J. WILKINS AND D. C. WEEKS, *Australian J. Sci. Research, Ser. B*, 4 (1951) 248.
5 D. J. ARNON, *Nature*, 184 (1959) 10.
6 F. LIPMAN, *Science*, 128 (1958) 575.
7 A. KYLIN, *Botan. Notiser*, 113 (1960) 49.
8 A. KYLIN, *Physiol. Plantarum*, 13 (1960) 148.
9 A. KYLIN, *Physiol. Plantarum*, 13 (1960) 366.

Light sensitivity of the mould *Thamnidium elegans**

Thamnidium elegans is a saprophytic mould which is most commonly found on dung, cold-stored meat and in the soil. Morphologically it is interesting because its asexual spores are either produced in large terminal sporangia or in smaller sporangioles borne at the ultimate ends of a system of dichotomous branches. The sporangia develop in the same way as those of *Phycomyces*; but it is only the stage between the first appearance of the sporangiophore and the production of a fruiting body that is to be considered here.

Hawker[1] has reported that the sporangia of *T. elegans* are produced only in the light, and in an attempt to study this effect, together with suspected disturbances in growth rate following illumination, a special time-lapse camera was built. This camera photographs every minute the mould growing at 22.5° inside a moveable incubator. The

* The work described in this paper was financed by an Agricultural Research Council Postgraduate Studentship, and this help is gratefully acknowledged.

photographic exposure was made with a red light with no transmission below 600 mμ to which the fungus shows no obvious response. The experimental illumination was provided by a focussed beam from a tungsten lamp. The humidity was kept constant at about RH 98% by growing the fungus in a transparent box containing a saturated solution of KH_2PO_4 and the box itself placed in the incubator.

It was confirmed that light induces the production of sporangia. It was found, however, that it is only the developing sporangiophore that is light-sensitive in this

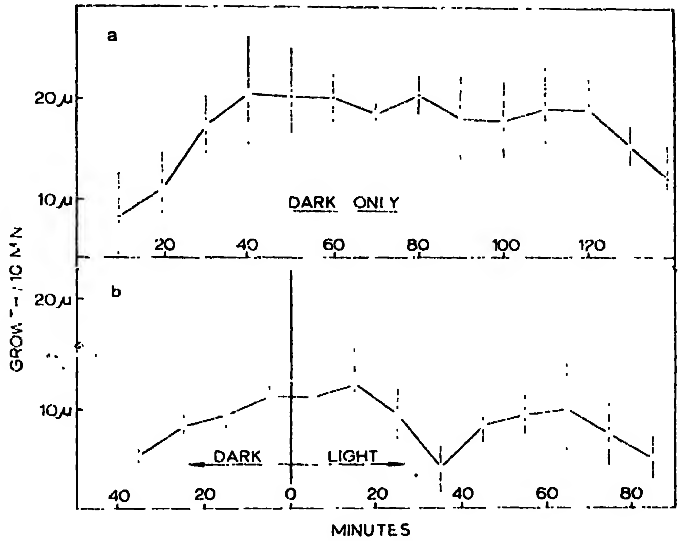

Fig. 1. Mean growth rate curves for stage I sporangiophore elongation in *Thamnidium*. The vertical lines represent twice the standard error.

respect and that it loses its sensitivity after about 50 min of upward growth. In unilluminated cultures sporangioles alone are produced.

It was also observed that the quantity of light shone on the developing sporangiophore influences the length of time that the sporangiophore elongates before a fruiting body is produced. An effect of this nature has been reported by Banbury[2] for *Phycomyces*. In *Thamnidium* the relationship between the quantity of illumination and the time it takes for the sporangiophore to fruit seems complicated and is more fully discussed in a paper in preparation for the *Trans. Brit. Mycol. Soc.*

Light also causes a temporary slowing of the sporangiophore growth rate. Fig. 1a shows the mean growth rate curve for several sporangiophores growing in the dark and Fig. 1b the mean growth rate curve for sporangiophores which appeared later and were illuminated. The lower mean growth rate for the illuminated sporangiophores is probably due to staling in the cultures. The time that it takes the minimum growth rate to develop is, like the lag phase for the growth rate response in *Phycomyces*[3], dependant on temperature. Lowering the intensity of the illumination by up to 80%, does not greatly alter the time for the minimum growth rate to develop. Unfortunately, however, this technique is not accurate enough to precisely determine the time when the growth rate minimum occurs at low light intensities.

It was early noticed that *Thamnidium* is positively phototropic. When the fungus growing out horizontally from an agar block in a petrie dish is illuminated from one

References p. 362

side, no marked phototropic response is observed until the growth rate has reached a minimum (Fig. 2a). If on the other hand lateral illumination is discontinued when the growth rate has reached a minimum there is no phototropism (Fig. 2b). This suggests that although the phototropic response is in some way associated with the light

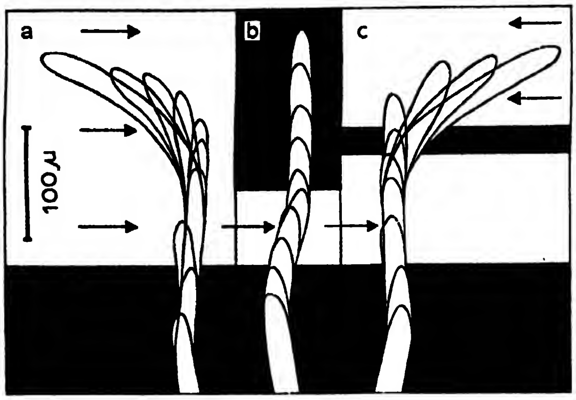

Fig. 2. Phototropic responses of stage I sporangiophores of *Thamnidium*. The sporangiophores are drawn at 10-min intervals.

induced slowing in growth rate, the two processes are separate. In an effort to show that the two responses are different, light from one direction was shone on the sporangiophore until the growth rate appeared to be at a minimum; then after 10 min in darkness, light from another direction was shone on the sporangiophore. There was a rapid curvature towards the second light (Fig. 2c).

It may be speculated from these experiments that the phototropic response is divided into two light-sensitive stages: (1) an un-orientated reaction which causes the cell to become sensitive to laterally asymmetric illumination and which is associated with a slowing of the growth rate, and (2) the phototropic response proper where laterally asymmetric illumination causes differences in the rate at which the cell wall is built with a resulting bend towards the light source. This second stage is associated with an increase in growth rate to about the original dark level.

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1 L. E. HAWKER, *The Physiology of Reproduction in Fungi*, Cambridge University Press, 1957, p. 44
2 G. H. BANBURY, *Handbuch der Pflanzenphysiologie*, Vol. 17, part 1, 1959, p. 530.
3 E. S. CASTLE AND A. J. M. HONEYMAN, *J. Gen. Physiol.*, 18 (1933) 385

Chloroplastenbewegung in polarisiertem Licht

Es ist bekannt, dass sich der plattenförmige Chloroplast von *Mougeotia* nach dem Licht orientieren kann, wobei er schwachem Licht die Fläche zukehrt, starkem dagegen die Kante.

Nur die Orientierung in schwachem Licht, also die Drehung in Flächenstellung, soll uns hier beschäftigen. Diese Schwachlichtbewegung ist ein ausgesprochener Induktionsvorgang, d.h. eine kurze Belichtung genügt, um in darauffolgender Dunkelheit die Bewegung ablaufen zu lassen. Die Induktion wird bevorzugt ausgelöst durch rotes Licht; der Induktionsvorgang unterliegt dem bekannten Hellrot-Dunkelrot-Antagonismus[1]. Das heisst, eine Induktion kommt durch Hellrot (HR; < 700 $m\mu$) zustande, anschliessend an HR gebotene Bestrahlung mit Dunkelrot (DR; > 700 $m\mu$) löscht dagegen die Induktion wieder aus.

Interessant wurden die Verhältnisse nun, als wir mit linear polarisiertem Licht zu induzieren versuchten und einen klaren Einfluss der Schwingungsrichtung feststellen konnten. Wir gehen stets aus von *Mougeotia*-Fäden, in denen die Chloroplasten in Profilstellung stehen, bestrahlen vom Profil her und stellen nach angemessener Dunkelzeit fest, in wieviel Zellen die Chloroplasten sich gedreht haben. Führen wir derartige Versuche mit polarisiertem Licht durch, so sehen wir eine Reaktion überwiegend in den Teilen des Fadens, die gerade senkrecht zur Schwingungsebene des Lichtes liegen. Lichtwirkungen, bei denen die Richtung der Schwingungsebene eine Rolle spielt, sind in den letzten Jahren verschiedentlich gefunden worden, aber stets handelte es sich um reine Blaulichtwirkungen, wie etwa beim Phototropismus von Pilzen oder bei der Polaritätsinduktion der *Fucus*-Zygote[2-4]. In unserem Falle dagegen handelt es sich um polarisiertes *Rot*licht, genauer HR, das im Rahmen des HR-DR-Reaktionssystems wirkt. Beim Phototropismus von *Phycomyces* diskutiert man die Möglichkeit, dass Fresnel'sche Reflexions-Verluste für die Unterschiede verantwortlich sind. Für unser Objekt reicht diese Erklärung nicht aus, da der Unterschied viel zu gross ist. Die Reaktionsgrösse in Prozent der reagierenden Zellen nimmt mit der Induktionsdauer zu, wenn die Schwingungsebene des hellroten Lichts quer zum Faden orientiert ist. Schwingt das Licht dagegen parallel zum Faden, so führt selbst hundertfache Induktionsdauer noch nicht zu nennenswerter Reaktion.

Wir müssen aus unseren Versuchen schliessen, dass die Photorezeptoren in der Zelle streng quer zum Faden orientiert sind. Dadurch entsteht ein Dichroismus, Absorption findet nur in der Schwingungsebene senkrecht zur Längsachse statt. In weiteren Versuchen belichten wir einen *Mougeotia*-Faden mit HR sowohl von der Kante als auch von der Fläche her hintereinander und beobachten, in welchem Masse dadurch die Induktion beeinträchtigt wird. Hierzu müssen wir einzelne Algenfäden in Glaskapillaren einsaugen und diese so montieren, dass wir die Kapillare und damit den Algenfaden beliebig um 90° drehen können. So lassen sich die Zellen mit exakt bekannten Intensitäten von verschiedenen Seiten bestrahlen. Wir verwenden HR (Schwingungsebene quer zum Faden orientiert), und bestrahlen von der Kante oder von der Fläche her. Bestrahlung allein von der Kante induziert Bewegung, wie zu erwarten.

Literatur S. 365

Bestrahlung allein von der Fläche hat keinen Effekt, wie zu erwarten. Bei Bestrahlung aus verschiedenen Richtungen bestimmt allein die *letzte* Richtung darüber, ob Reaktion eintritt oder nicht. Offenbar können wir also die Induktion löschen, wenn wir anschliessend an die Kantenbestrahlung noch die Fläche bestrahlen. Das soll nun quantitativ erfasst werden. Die induzierende HR-Bestrahlung von der Kante her halten wir konstant = 1 min, die darauffolgende HR-Bestrahlung von der Fläche mit gleicher Intensität variieren wir in der Dauer. Dabei finden wir, dass bereits 1/2 minutige Nachbestrahlung von der Fläche die 1-min Induktion weitgehend auslöscht.

Wir können aber die Induktion noch auf andere Weise unwirksam machen, ebenfalls durch eine HR-Bestrahlung, die gewissermassen „falsch" gegeben wird. Wir induzieren zunächst wie üblich mit HR von der Kante her (Schwingungsebene quer orientiert), und bestrahlen anschliessend aus der gleichen Richtung, aber nun mit längsorientierter Schwingungsebene. Überraschenderweise ist eine solche Nachbestrahlung nicht indifferent, sondern setzt die Wirkung der vorausgegangenen Induktion erheblich herab. Wir führen auch diesen Versuch quantitativ durch, indem wir die Dauer der Nachbestrahlung systematisch variieren und erhalten ganz ähnliche Resultate wie im vorigen Versuch. Wir können also eine HR-Induktion wieder löschen, wenn wir mit dem *gleichen* HR nachbestrahlen, entweder aus der falschen Richtung, nämlich von der Fläche, oder mit falsch orientierter Schwingungsebene, nämlich parallel zum Faden. Die antagonistische Wirkung einer Flächenbestrahlung ist stärker als die einer längsschwingenden Kantenbestrahlung.

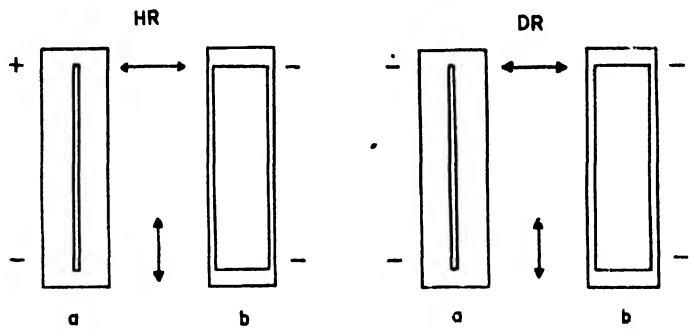

Fig. 1. *Mougeotia*-Zelle mit Chloroplast in Kanten- (*a*) oder Flächenstellung (*b*). Bestrahlung senkrecht zur Papierebene. Doppelpfeil: Schwingungsrichtung. HR = Hellrot, DR = Dunkelrot. + = Induktion; – Induktionslöschung (wenn auf Induktion folgend).

Wir wollen uns nun noch die Frage vorlegen, ob und in welcher Weise die antagonistische DR-Wirkung, also die Löschung der HR-Induktion durch DR, von der Schwingungsrichtung abhängig ist. Wir induzieren mit HR (Schwingungsebene quer zum Faden orientiert) und bestrahlen mit DR nach, dessen Schwingungsebene entweder quer oder längs orientiert ist. Verwenden wir dabei steigende Dosen DR, so erhalten wir Kurven zunehmender Induktionslöschung. Querschwingendes DR ist zwar etwas stärker wirksam als längsschwingendes, aber die Unterschiede sind doch recht gering, verglichen mit den Verhältnissen bei der Induktion durch HR. Wir können nun auch hier von der Möglichkeit Gebrauch machen, einzelne Fäden von verschiedenen Seiten her zu bestrahlen. Wir induzieren mit HR von der Kante und bestrahlen anschliessend mit DR, entweder ebenfalls von der Kante, oder von der Fläche (alle Bestrahlungen

mit quer zum Faden orientierter Schwingungsebene). Mit steigenden Dosen DR erhalten wir wieder Wirkungskurven, aus denen wir entnehmen können, dass Induktionslöschung sowohl von der Kante als auch von der Fläche her möglich ist und dass die Wirksamkeit in beiden Fällen in der gleichen Grössenordnung liegt.

Während also die Photorezeptoren in der HR-absorbierenden Form streng orientiert zu sein scheinen, gibt es für den DR-absorbierenden Zustand keinen Hinweis auf eine strukturelle Ordnung. In der HR-absorbierenden Form des Photorezeptors ist darüberhinaus die Schwingungsrichtung nicht nur quantitativ, sondern auch qualitativ von Bedeutung für die Reaktion.

Eine zusammenfassende Übersicht über alle Resultate gibt das Schema der Fig. 1. Vergleiche auch die ausführliche Darstellung der Ergebnisse[5].

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[1] W. HAUPT, *Planta*, 53 (1959) 484.
[2] E. S. CASTLE, *J. Gen. Physiol.*, 17 (1934) 751.
[3] L. F. JAFFE, *Exptl. Cell Research*, 15 (1958) 282.
[4] W. SHROPSHIRE, *Science*, 130 (1959) 336.
[5] W. HAUPT, *Planta*, 55 (1960) 465.

Orientation of cell growth by polarized radiation

Four years ago it was reported that *Fucus* zygotes germinate parallel to the vibration plane* after exposure to plane polarized visible light[1]. Further study led to the conclusion that this 'polarotropic' response is a variant of the general phenomenon of phototropism and is mediated by a periclinal orientation of dichroic photoreceptor molecules; *i.e.* the axes of maximum molecular absorption lie parallel to the nearby surface of the zygote[2]. Similar polarotropic responses were also discovered in the spores of a moss, a fern, and the imperfect fungus, *Botrytis*[3].

In this paper, we report proof that the polarotropic response of *Botrytis* spores is also a variant of phototropism mediated by an orientation of dichroic photoreceptor molecules with respect to the nearby surface. However, in this case it is shown that the axes of maximum absorption of these molecules, unlike those in *Fucus*, are anticlinal; *i.e.* they are perpendicular to the nearby surface. We also report a somewhat less rigorous analysis of the polarotropic responses of the spores of the fern, *Osmunda*, indicating periclinal photoreceptors similar to those in *Fucus*. Again, we report a preliminary study of the polarotropic responses of the spores of the moss, *Funaria*. This indicates a situation which is anomalous not only in a high responsiveness to red light, but also in the presence of at least two different tropic photoreceptor molecules, one of which is *un*oriented while the other is anticlinal. Finally, we report a qualitatively new aspect of the tropic responses of *Botrytis* spores to light, the phenomenon of *centering*.

(1) A preliminary study showed strong tropic responses of *Botrytis* spores to blue

* *In the biological literature, the plane of vibration is the plane of the E-vector.*

References p. 367

light. However, the spores germinate at random when exposed to polarized radiation in the red, various portions of the infrared, and the V.H.F. radio frequency region, even when this radiation is applied in intensities up to 10^6 to 10^8 times those effective in the blue.

(2) Proof that the photoreceptors in *Botrytis* spores are highly dichroic and sharply anticlinal is as follows: Spores were exposed to various doses of polarized blue light; in companion experiments spores were exposed to the same light doses applied as 100% gradients* of unpolarized light across each cell; in both series the direction and degree of orientation were determined. The spores tend to germinate *parallel* to the plane of vibration in the first series and from the *bright* end of the applied gradient in the second. In both series, the degree of orientation rises in an S-shaped way with dose, going from 10% to 60% to 90% as the dose rises from 10^2 to 10^3 to $3 \cdot 10^5$ ergs/mm². Indeed, in the latter dose range (from 10^3 to $3 \cdot 10^5$ ergs/mm²) no significant difference at all is found in the degree of response to the two types of illumination; thus polarized light corresponds in its effect to an approximately 100% gradient. Now it is easily shown that an inference of highly dichroic and anticlinal photoreceptors will explain these observations while an inference of weakly dichroic and/or periclinal receptor molecules will not.

Positive confirmation that the receptors are anticlinal is furnished by the response of the spores to unilateral unpolarized illumination. Anticlinal receptors in a sphere under such illumination will absorb most light neither at 0° nor at 180° (with respect to the direction of the light source taken as 0°), but rather at about 90°. In conformity to this, germination under unilateral illumination is found to occur most frequently at about 115°.

Moreover, all other alternatives can be ruled out. Differential *scattering* would effect germination perpendicular rather than parallel to the vibration plane. Differential *reflection* at the cell-medium interface is estimated to produce gradients of less than 0.5% within the cells. Now, orientation as a function of the size of the gradient at a dose of $2 \cdot 10^4$ ergs/mm² was directly measured over the whole range from 0 to 100% gradients. From this result it can be interpolated that a 0.5% gradient effects about 1% orientation at a dose of $2 \cdot 10^4$ ergs/mm²; yet this dose of polarized light is found to cause an orientation of 90%. Hence the effect of differential reflection is negligible. Measurements of the polarotropic response of spores illuminated via air or oil rather than water confirm this inference. For in the former media, reflection at the cell surface is greatly increased; yet polarotropic orientation decreases in them. Finally, *polarizing screen pigments* concentrated enough to produce gradients of the order of 100% would be grossly obvious. Since the cells appear hyaline, such screening must be negligible. We know of no other alternative explanation of polarotropism in *Botrytis* which seems worthy of serious consideration.

(3) Tropic *centering* in *Botrytis* is our reference to the following observation: spores were unilaterally illuminated with unpolarized light. At all stimulating doses except the highest used, thus from 10^2 to 10^5 ergs/mm², the resultant distribution of outgrowth

* Such gradients were achieved as follows: a pattern of very thin, *sharply bounded*, and opaque stripes of chromium was formed upon a quartz slide. Cells were fixed directly upon this striped substratum and covered with a clear nutrient medium. They germinated normally and in random directions in the dark. When illuminated from below only, any cell which chanced to lie upon the boundary of a chromium stripe was so sharply shadowed as to be subjected to a 100% gradient.

directions has two peaks at about ±115°. However, at 10^6 ergs/mm² the response changes dramatically. The distribution continues to show two peaks at ±115° but a third, large and *distinctly separate* maximum appears at 180°. We suggest that this new peak does not arise from any change in the photoreceptors but rather from a spreading out, an overlapping and an addition of the growth stimuli from the foci at ±115°. The rear pole of each cell, *i.e.* 180°, would thus be the direction in which this addition produces a new maximum.

Older observations in mosses and in the Fucaceae of a shift in the direction of preferred germination from the 'subequatorial' region between 90 and 135° to the rear pole probably represent less obvious examples of such centering.

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1 L. JAFFE, *Science*, 123 (1956) 1081.
2 L. JAFFE, *Exptl. Cell Research*, 15 (1958) 282.
3 E. BÜNNING AND H. ETZOLD, *Ber. deut. botan. Ges.*, 71 (1958) 304.

Effect of ionising radiation on oxidation processes in plants

The effect of ionising irradiation on plant organisms is to produce biochemical changes as a result of damage to general metabolic processes. The investigation of these changes is of essential interest since primary biochemical changes can be detected before cell structural damage is evident. Many disruptions of biochemical reactions are characterized by intensive oxidation. Biochemical changes connected with the formation of toxic substances in the irradiated organism, *e.g.* lipid peroxides, are of great interest[1].

It was shown earlier that the activity of the enzyme system oxidizing unsaturated fatty acids (lipoxidase) in various irradiated plant leaves (wheat, barley, bean, soya and peas) increases 30 to 50% in the course of the 24 h following irradiation[2-4]. Investigation of chemical changes after irradiation in free and bound lipids of bean leaves has shown that in the free lipid fraction of irradiated leaves the peroxides are present in much greater quantity than in non-irradiated ones[5,6]. The purpose of this investigation is to study changes in free lipid composition after plant tissue irradiation.

Bean ("*Latvia*") and barley ("*Viner*") seedlings 10–14 days old which had been grown in a hot-house, were exposed to irradiation. Before irradiation, plant seedlings were dug out with every possible precaution and placed into beakers filled with tap water. The soil was previously washed off the plant roots. The irradiation was carried out with the help of an IRU-I apparatus (Industrial X-ray Unit-I), the irradiation intensity being 595 r/min and the focus distance being 22 cm. The diameter of the crown of the leaves did not exceed 9–11 cm. The material was lyophilized 2, 4, 6 or 24 h after the irradiation. The isolation of free and bound lipids from irradiated plant

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References p. 370

leaves was carried out by means of extraction with petroleum ether at room temperature (after Zirm, Pongraz and Polesovsky[7]). The determination of peroxide in free and bound lipids was carried out by the method of Glavind and Hartmann[8] and for the paper chromatographic separation of free lipids and unsaturated fatty acids, the methods of Spitery[9] and Alimova[10] were followed. Pure unsaturated fatty acids were obtained by fractionating the fatty acids obtained by plant oil and animal fat saponification according to the method of Svern and Parker[11], the unsaturated fatty acids being separated by paper chromotography. Further separation of these acids was accomplished by means of the low-temperature fractional crystallization method[12].

After the leaves had been irradiated by 1000 to 10,000 r, sharp changes in the lipid content were observed (Table I). As can be seen from these experiments (2 or 3 parallel determinations were done), the free lipid fraction in irradiated seedling leaves contained amounts of peroxides 2 to 6 times as great as those of non-irradiated ones. The average weight of dry material in each experiment did not exceed 0.1 to 0.3 g. The measurement of peroxides in the bound lipid fraction showed that a quite negligible amount of peroxide is formed on irradiation, *i.e.* 0.16 0.42 mequiv./kg dry weight

TABLE I

EFFECT OF IONIZING IRRADIATION ON PEROXIDE CONTENT IN FREE LIPID FRACTION OF SEEDLING LEAVES

Time after exposure (h)	*Irradiated or non-irradiated leaves*	*Peroxide content* mequiv./kg of dry weight	%
Bean			
1/6	Non-irradiated	2.450	100
	1000 r	5.970	243.7
	10000 r	10.640	430.2
1/4	Non-irradiated	1.730	100.0
	1000 r	2.270	145.2
	10000 r	3.930	226.7
2	Non-irradiated	1.537	100.0
	1000 r	2.649	172.2
	10000 r	2.846	182.7
2	Non-irradiated	3.793	100.0
	1000 r	5.66	149.3
4	Non-irradiated	1.730	100.0
	1000 r	2.250	145.0
	10000 r	4.720	261.2
24	Non-irradiated	0.411	100.0
	1000 r	2.300	559.0
24	Non-irradiated	8.425	100.0
	10000 r	13.220	157.0
Pea			
1/4	Non-irradiated	2.960	100.0
	10000 r	7.200	243.0
Barley			
2	Non-irradiated	4.300	100.0
	10000 r	5.400	120.9
2	Non-irradiated	0.700	100.0
	10000 r	0.900	125.0
6	Non-irradiated	16.600	100.0
	10000 r	83.800	505.0
24	Non-irradiated	0.700	100.0
	10000 r	2.500	375.0

3–5% on the control). Nevertheless, the peroxides in the bound lipid fraction after irradiation undergo changes similar to those of the peroxides in the free lipid fraction. Table I also indicates that the absolute amount of peroxide is not always constant in the same plant. The highest peroxide content was observed in 10-day seedling leaves of the spring–summer period. The amount of peroxide in the leaves was considerably lower in both control and irradiated plants in the autumn–winter season. In this period less intensive metabolism and a slower growing rate were also observed.

The separation of the free lipid fraction from bean leaves by paper chromotography before and after irradiation showed that irradiation of these leaves with 20000 r had produced considerable changes in the unsaturated fatty acid content of this fraction. (Fig. 1). After 4 h the effect of the ionizing irradiation on almost all unsaturated fatty acids disappeared from the free lipid fraction extract of irradiated plants.

To solve the problem of the nature of peroxide formation in irradiated leaves a

TABLE II

EFFECT OF IONIZING IRRADIATION ON PEROXIDE CONTENT OF FREE LIPID FRACTION OF STEAMED SEEDLING LEAVES

Time after exposure (h)	*Irradiated, non-irradiated or steamed bean leaves*	*Peroxide content*	
		mequiv./kg dry weight	*%*
1/6	Non-irradiated	2.450	100.0
	1000 r	5.970	243.7
	Steamed + 1000 r	1.140	46.5
24	Non-irradiated	6.485	100.0
	Steamed	4.[illegible]60	71.9
	Steamed + 10000 r	1.829	28.2
24	Non-irradiated	5.423	100.0
	Steamed	4.841	89.3
	Steamed + 10000 r	4.803	79.4
24	Non-irradiated	106.4	100.0
	10000 r	161.0	150.0
	Steamed + 10000 r	62.9	59.1

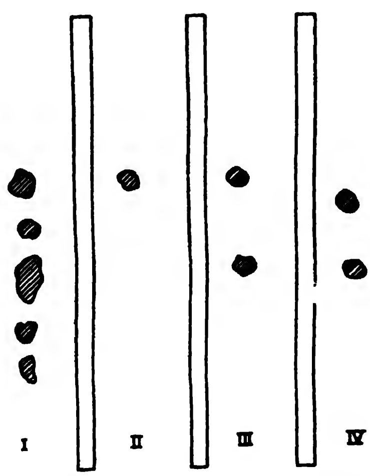

Fig. 1. The pattern of unsaturated fatty acids in the free lipid fraction of bean leaves on paper chromatograms. I. Control; II. Irradiated with 20000 r (after 4 h); III. Irradiated with 20000 r (after 24 h); IV. Mixture of pure linoleic and oleic acids.

References p. 370

number of experiments were carried out in which the seedling lipoxidase was inactivated by steam heating for 7 to 10 min. The activity of steamed leaf lipoxidase was measured manometrically in the Warburg apparatus and it was found to have been fully inactivated. The irradiation experiments on steamed bean leaves were then performed, the non-irradiated fresh seedling leaves being the controls. Steamed leaves, irradiated leaves and leaves first steamed and then irradiated with 10000 r were taken for the experiment.

Table II shows that the irradiation of leaves in which the lipoxidase was inactivated by steam did not result in peroxide content increase, while irradiation of non-steamed control leaves brings about a sharp increase in lipid peroxide content. The data indicate that with the increase of lipoxidase activity[2-4], the quantity of lipid peroxide increases after quite small doses of radiation (1000 r). As compared with non-irradiated leaves, the peroxide content of the free lipid fraction of irradiated plants increases approximately 2 to 6 times. The results of the separation of the free lipid fraction by paper chromotography also indicate considerable changes in its constitution after irradiation.

Thus, the biological effect of ionizing radiation on plant seedlings can be seen in lipoxidase activation, intensification of oxidation in lipid fraction and accumulation of lipid peroxides which may play an important role in X-ray damage to the living plant organism.

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1 P. DUBOULOZ AND J. DUMAS, *Compt. rend soc. biol.*, 148 (1954) 705.
2 E. V. BUDNITSKAYA, I. G. BORISOVA AND A. G. PASSINSKIY, *DAN*, 120 (1) (1958) 1407.
3 E. V. BUDNITSKAYA AND I. G. BORISOVA, *IV Intern. Congr. Biochem.*, 18 (1958) 8.
4 E. V. BUDNITSKAYA AND I. G. BORISOVA, *Sbornik Radiobiologia*, Izd. AN SSSR, 1957.
5 E. V. BUDNITSKAYA, I. G. BORISOVA AND A. G. PASSINSKIY, *Biochimia*, 23 (6) (1958).
6 E. V. BUDNITSKAYA AND I. G BORISOVA, *DAN*, 126 (1) (1959).
7 K. Z. ZIRM, A. PONGRATZ AND W. POLESOFSKY, *Biochem. Z.*, 326 (6) (1955).
8 I. GLAVIND AND S. HARTMANN, *Acta Chem. Scand.*, 9 (3) (1955) 497
9 I. SPITERY, *Bull. soc. chim biol.*, 36 (1954) 355.
10 E. K. ALIMOVA AND G. D. BOLGOVA, *Biochimia*, 22 (1956) v.3.
11 D SWERN AND W. PARKER, *J. Am. Oil Chemists' Soc.*, 30 (1) (1953) 57.
12 A. FRENCEL, C. BROWN AND B. STONEBURNER, *J. Am. Chem. Soc.*, 61 (1941) 1483.

The effect of light intensity on growth and development of *Gladiolus*

Introduction and technique

As well as studies under controlled conditions, we carry out in our laboratory field experiments with variation of a single environmental factor. In photoperiodic field experiments, variation is restricted to daylength by simultaneously covering long- and short-day plots, and supplying weak artificial illumination to the long-day plot[1]. In other experiments, natural daylight intensity is varied by permanent screening. This has been done with barley[2,3], sugar beet[2,4], and *Gladiolus*. The present paper discusses effects of artificial weakening of light on growth, efficiency of solar energy conversion, and formative behaviour in *Gladiolus**, recorded in 1959 by periodic harvests as used in[1–4].

Four light intensities, *viz.*, about 12, 37, 75 and 100% of natural daylight were applied, the first three produced by metal gauze top screens with side screens of similar type; top and side screens were lifted as required during growth. Each unit covered 2 × 2 m, admitting 100 plants. A double row in all units was outside the screen (or field in case of 100% light).

Each corm was weighed before the experiment, those between 16 and 27 g being used for planting. All corms were individually numbered, and the four fields each planted with 100 corms of very similar weight distribution. Dry weight was recorded from samples of seven size classes; it varied between ~ 6 and 9 g/corm, dry weight percentage from 36 to 33, with the lower values in the heavier corms. Corms were planted April 23, 1959, emergence was about 11 to 14 May, first harvest taken June 22. Five plants from each unit were taken at each harvest, and their parts weighed separately. Dry weight was determined for all parts of the average plant; fresh weight of all parts of each plant reduced to dry weight by dry weight percentages of the analysed plant, and averaged.

Dry weight

Fig. 1 shows the successive development of various plant organs during the season in relation to light intensity. Root weight is low in all cases. The old corm has much the same dry weight (~ 2 g) during the entire season at all light intensities, with only a slight decrease at late dates. Obviously, a considerable depletion, from ~ 7 g to ~ 2 g took place before June 22. At the lowest light intensity (~ 12% of natural daylight) there is no appreciable gain in dry weight by June 22, at higher intensities there is. The plant consists chiefly of old corm and leaves. The subsequent increase (July 13) is mainly in leaves, while stem dry weight begins to develop, especially at high light intensities. This continues (Aug. 3), total dry weight still increases, and, at high light intensities, stem dry weight becomes the main component, while leaf dry weight decreases somewhat.

* A cultivar of *Gladiolus (hybr.) gandavensis* v. Houtte, the "ordinary large garden gladiolus" (ref. [5], p. 366).

References p. 378

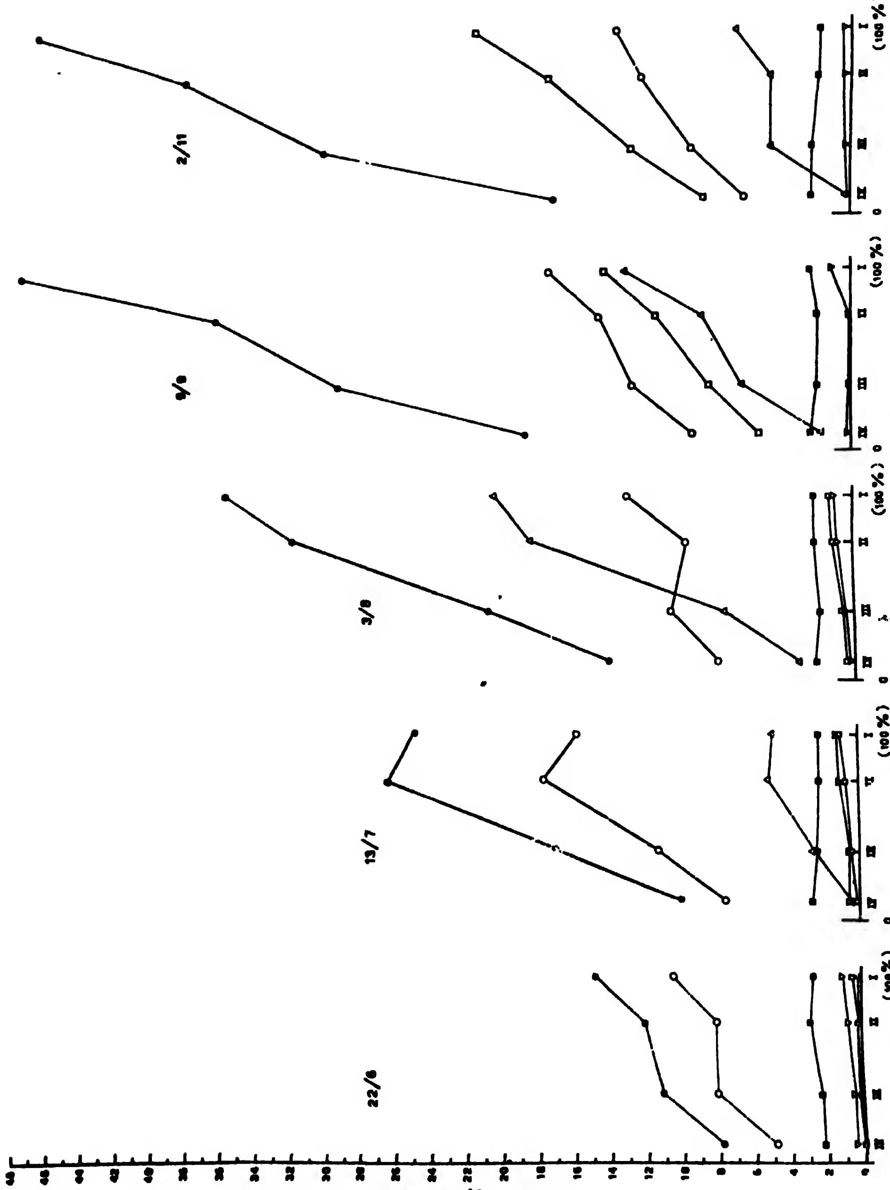

Fig. 1. *Gladiolus*. Field experiment with artificial shading. Dry weight development at choice of successive harvests. ○ leaves, △ stem, ▽ roots, ■ old corm, □ new corm, ● total. Light intensities I, II, III, IV: 100, ~ 75, ~ 37, ~ 12 % of daylight respectively

The slight decrease in total dry weight from 75 to 100% light, observable at 13.7, may be attributed either to the rather small number of 5 plants per harvest or, more likely, to the fact that the 100% had temporarily suffered from drought more than the others.

Total dry weight reaches its maximum at all light intensities at about Sep. 9 (see also Fig. 2); after 3.8, leaf dry weight remains much the same while stem dry weight strongly decreases (plants did not produce seeds) in favour of the new corm, the dry weight curve of which crosses that of the leaves in much the same way as stem dry weight did earlier. The new corm eventually becomes the main dry weight component at all light intensities. It should be stressed that the curves represent absolute weight, not percentage of total. The dry weights of the major components at their peak development (Fig. 3) show mutually similar dependencies on light intensity, with apparently steepest slope in the stem.

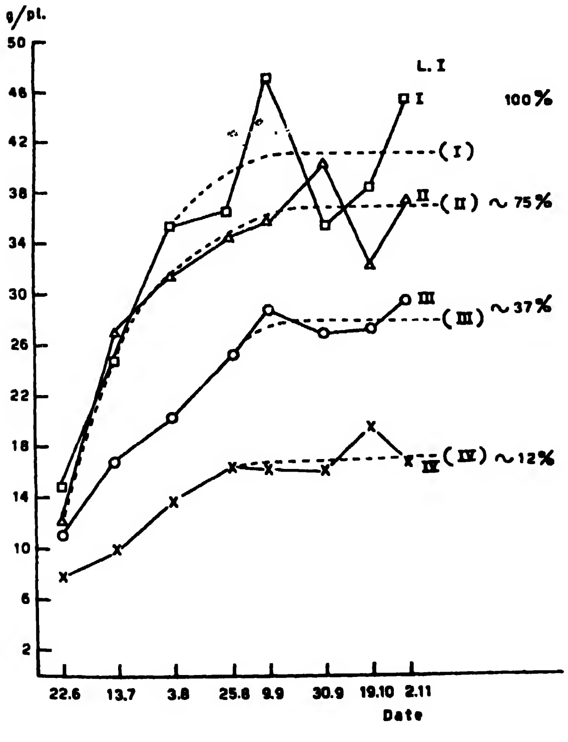

Fig. 2. Development of total dry weight at successive harvests, and "idealized" final values.

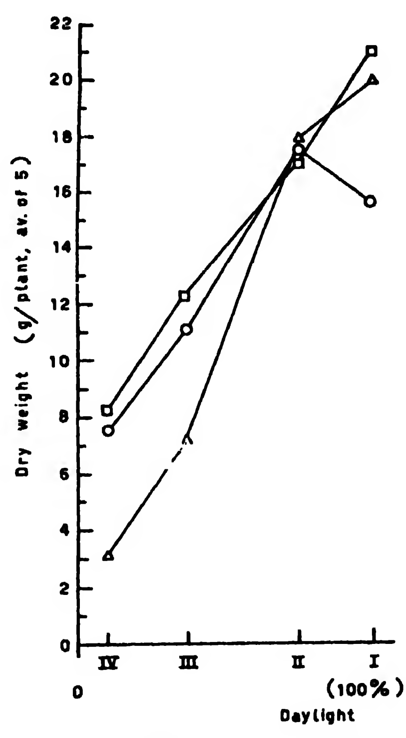

Fig. 3. Dry weights of leaves ○ (13.7), stem △ (3.8), and new corm □ (2.11) at their peak development in relation to light intensity.

Efficiency of solar energy conversion

Only total dry weight has so far been used for efficiency determinations, 5 plants covering $5/100 \times 4$ m², *i.e.* 2000 cm². Calories solar radiation per cm² p. day were obtained from records of the Physics Department of this University. Chemical energy of entire plant was assumed to be 4 kcal/g. At 12 and 37% daylight, increase in dry

References p. 378

weight was about linear between 22.6 and 25.8; at 75 and 100% daylight a steeper initial part was indicated (Fig. 2). Maximal dry weights, reached about 9.9, idealized from values recorded 9.9 to 2.11 are about 41, 37, 28, and 17 g p. plant for 100, 75, 37 and 12% light respectively (Fig. 2). Efficiencies, based on photosynthetically active radiation (assumed 50% of total) are shown in Table I. In the period of most active growth (22.6 to 25.8) they decrease from ~ 0.05 to < 0.02 with increasing light intensity (and increased yield). The early period (11.5 to 22.6) is inefficient, like that after 25.8, decreasing efficiency values if included (0.023 to 0.010 for period 11.5 to 2.11 over the range of light intensities, see Table I and Fig. 2).

TABLE I

EFFICIENCY OF SOLAR ENERGY CONVERSION IN *Gladiolus* (1959)
Photosynthetically active radiation only, per unit soil area

Date (period)	Light intensity 12%	37%	75%	100%
11.5 – 22.6	0.005	0.009	0.005	0.0066
22.6 – 13.7			0.042	0.021
22.6 – 3.8			0.027	
22.6 – 25.8	0.049	0.027	0.021	0.017
22.6 – 9.9	0.043	0.026	0.019	0.015
11.5 – 9.9	0.028	0.020	0.014	0.012
11.5 – 2.11	0.023	0.016	0.011	0.010

Some morphogenetic features

Along with energetic effects as discussed, differences in light intensity induce morphogenetic changes. Fig. 4 shows representative plants in successive stages. Fig. 5 shows successive leaves, and stem at 10.8. At early stages the stem is still small, the apparent "stem" consists wholly of the sheaths of the visible leaves, tightly surrounding each other, and inserted on the old corm. At lower light intensities, both sheath and lamina of the leaves are more elongated than at higher light intensities. The boundary between sheath and lamina is oblique, and difficult to define sharply for measuring purposes. The following refers to total leaves.

The length: width relation (L/B) is about the same for leaves 4 and 5, and somewhat lower for leaf 3. Average L/B-value of leaves 3, 4 and 5 (leaf 1 is the first leaf with definite lamina), established at each harvest, decreases with light intensity from ~ 35 at the lowest to ~ 18 at the highest light intensity (Fig. 6).

Stem length is much less influenced by light intensity than is stem dry weight (Fig. 7). For instance, at 3.8, stem dry weight at 100, 75, 37, and 12% light was 100, 90, 36, and 15%; stem length was 100, 103, 110, and 96% respectively. Flowering is much reduced at low light intensities.

Fig. 8 shows 1 : L/B or B/L, and the final "idealized" dry weight in relation to light intensity. In the light intensity range studied the curves are very similar,

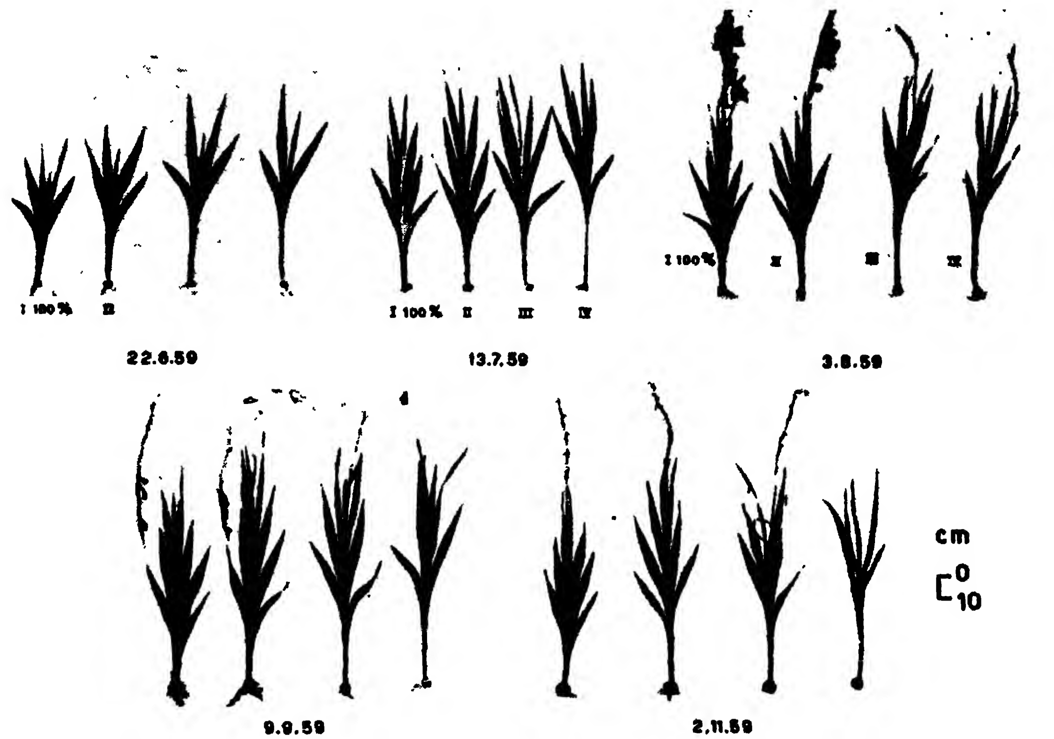

Fig. 4. *Gladiolus*. Plants of field experiment with artificial shading. Choice of successive harvests. Four light intensities each, decreasing from left to right. (Light intensities I, II, III, IV: 100, ~75, ~37, ~12% daylight).

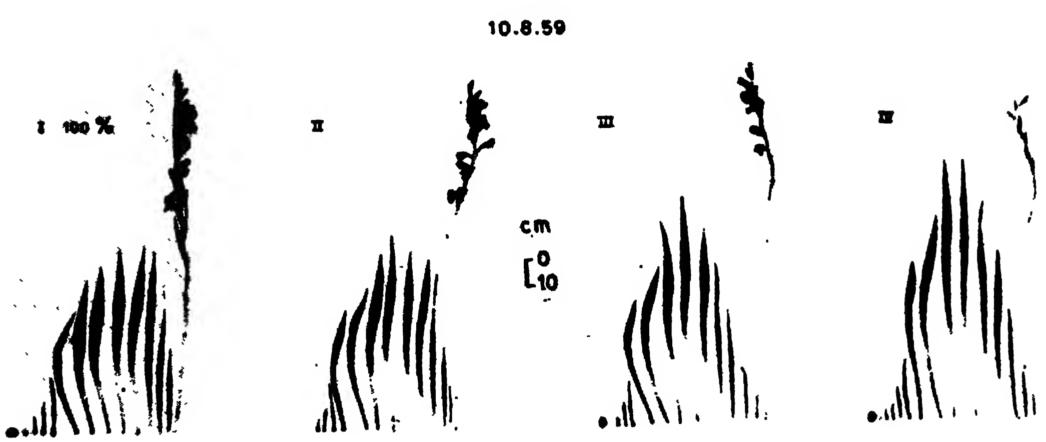

Fig. 5. Analysis of plant at each light intensity, decreasing from left to right, harvested 10.8.59.

References p. 378

suggesting a close relationship between the formative effect of light intensity on leaf shape and the energy level of the plant, as in lettuce under certain conditions[6]. This morphogenetic effect of high light intensities (see also ref.[7,8]) is interesting beside those of low amounts of photosynthetically active and inactive radiation[9–12].

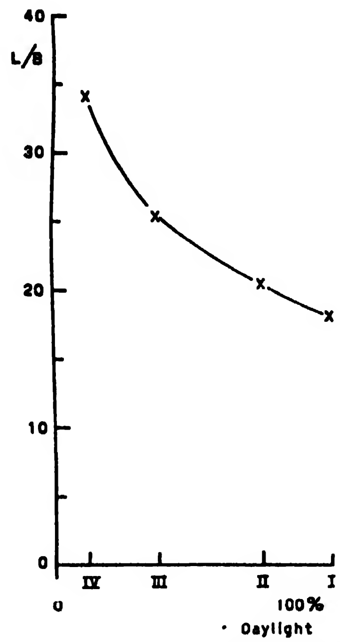

Fig. 6. Average L/B relation of 3rd to 5th leaf in relation to light intensity. Average of records from 25.8 to 2.11.

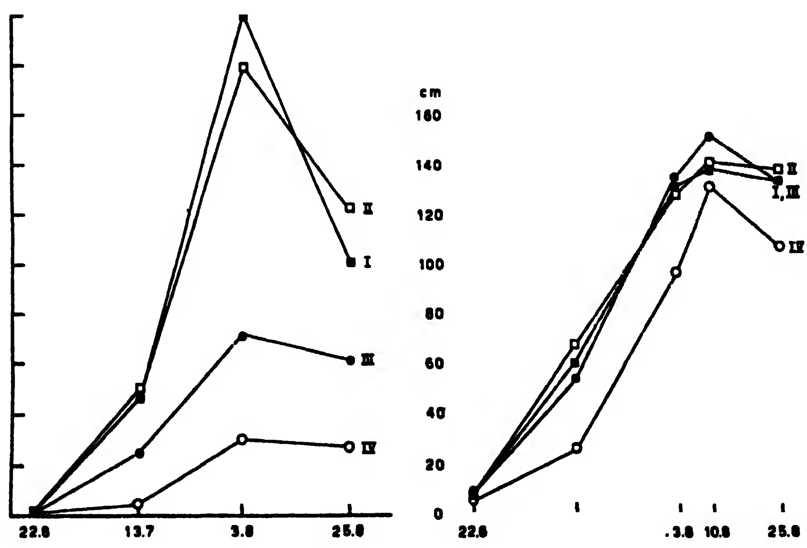

Fig. 7. Stem dry weight (left) and stem length (right) in relation to light intensity at successive dates (Light intensities: I, II, III, IV: 100, ~75, ~37, ~12% daylight).

Leaf area (LA, green part of stem included, Fig. 9) is less dependent on light intensity than leaf shape and dry weight (Fig. 8); the curves strongly resemble those of stem length (Fig. 7) in that 37 to 100% light are close together while only 12% is somewhat lower.

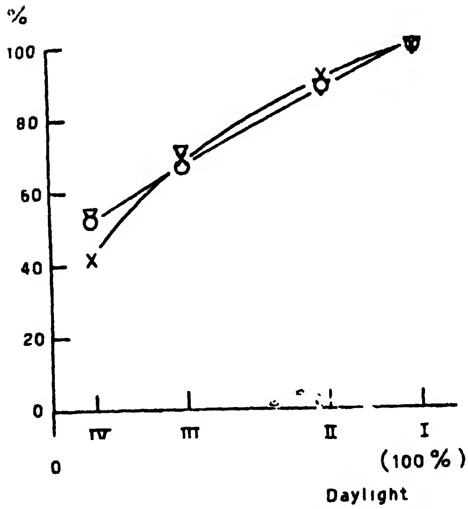

Fig. 8. Relative effects of light intensity on final dry weight ("idealized") in %, and on leaf shape (B/L × 100 in %) calculated from Fig. 5). × final dry weight, O 4th leaf, average figures 25.8 to 30.9 included, ∇ 3rd to 5th leaf, average figures 25.8 to 2.11 included.

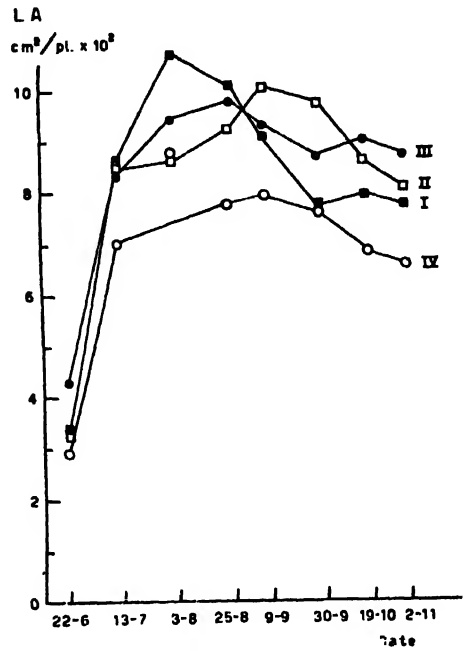

Fig. 9 Leaf area as dependent on season and light intensity (I–IV, see legend Fig. 7).

A major formative effect in a plant is the distribution of matter over its various organs. At 3.8, the stage of major stem development, leaf percentage in dry weight in 12, 37, 75, 100% light was 56, 50, 29.5, 35.5, stem percentage 22.7, 35.4, 57.6, 57.2. At 2.11, the final date, leaf percentage in dry weight in 12, 37, 75, 100% light was 36, 30, 31.5, 28.5, new corm percentage was 49, 41.5, 45.5, 45.8 respectively. The relative stem development thus seems much more light intensity dependent than the relative new corm development, a "useful" adaptation for persistence under unfavorable light conditions.

Leaf area ratio (LAR) and Net assimilation rate (NAR)

LAR-values (cm²/g) vary from ~ 70 to ~ 20 (Fig. 10) with highest values at the lowest light intensity and early in the season. Unlike dry weight, LAR seems "saturated" around 75% light, at this intensity LAR was remarkably constant ~ 30 during the season.

NAR (g/cm²/day) is high at the beginning of the season (up to ~ 10^{-3}, Fig. 11A), and declines in two major steps (around 13.7 and 9.9) to ultimate low values. NAR over the entire season is fairly linear with light intensity (Fig. 11B).

References p. 378

Leaf area index (LAI, cm^2 leaf area/cm^2 covered surface) is not well defined in a plant of the *Gladiolus* type. Taking surface area as area available for each plant (400 cm^2) it is directly related to the LA-values in Fig. 9, and varies from ~ 0.7 to ~ 2.7

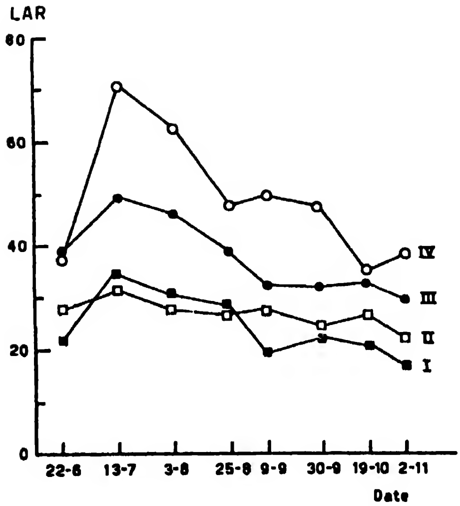

Fig. 10. Leaf area ratio (LAR, cm^2/g) as dependent on season and light intensity (I–IV, see legend Fig. 7).

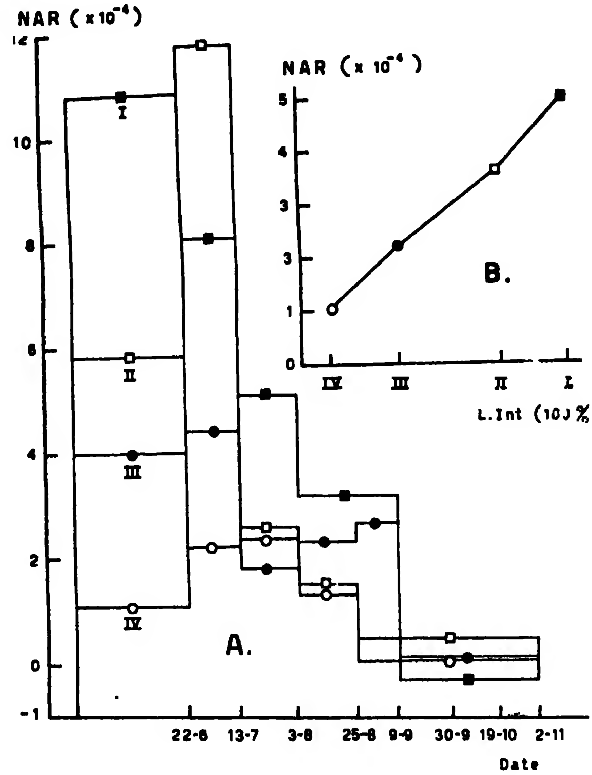

Fig. 11. A. Net assimilation rate (NAR, g/cm^2·days) as dependent on season and light intensity. B. Average values of NAR over entire season as related to light intensity. (I–IV, see legend Fig. 7).

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1 E. C. WASSINK AND J. A. J. STOLWIJK, *Mededel. Landbouwhogeschool Wageningen*, 53 (1953) 99.
2 M. S. KAMEL, *Mededel. Landbouwhogeschool Wageningen*, 58 (8) (1958) 1.
3 M. S. KAMEL, *Mededel. Landbouwhogeschool Wageningen*, 59 (5) (1959) 1.
4 M. S. KAMEL, unpublished.
5 A. J. HERWIG, *Practische Tuin-Encyclopaedie*, Arnhem, 1937
6 J. BENSINK, *Proc. Koninkl. Ned. Akad. Wetenschap.*, C, 61 (1958) 89.
7 E. C. WASSINK AND L. E. Æ. WASSINK-VAN LUMMEL, *Rept. 13th Intern. Horticult. Congr., 1952, London*, 1953, p. 969.
8 E. C. WASSINK, P. J. A. L. DE LINT AND J. BENSINK, *Intern. Symposium on photoperiodism in plants and animals, and related phenomena, Gatlinburg, Tennessee, U.S.A.*, 1957, p. 111.
9 E. C. WASSINK, C. M. J. SLUYSMANS AND J. A. J. STOLWIJK, *Proc. Koninkl. Ned. Akad. Wetenschap.*, 53 (1950) 1466.
10 E. C. WASSINK AND J. A. J. STOLWIJK, *Ann. Rev. Plant Physiol.*, 7 (1956) 373.
11 E. C. WASSINK, J. BENSINK AND P. J. A. L. DE LINT, *Symposium on Photoreceptors, Rept 2nd Int. Photobiol. Congr. Turin, Italy, 1957*, p. 196.
12 E. C. WASSINK AND W. SYTSEMA, *Mededel. Landbouwhogeschool Wageningen*, 58 (7) (1958) 1.

[*] 205th Communication; in part 76th Communication on photosynthesis.

Growth of *Lemna minor* as influenced by light and kinetin

Light, in low doses, promotes the growth of *L. minor* and cannot be replaced in this function by sugars, amino acids or yeast extract[1]. Only the effect of kinetin equals that of short exposures to red light[2]. The results of some experiments on the effects of alternating red and infrared light, of different doses of light and of kinetin, and on the ability of kinetin to induce growth in darkness will be reported here.

L. minor was cultivated in a sterile medium containing nutrient salts; 1% sucrose and 50 mg/l casein hydrolysate. The multiplication rate and the mean area of the full-grown frond were taken as criteria for growth.

The effect on the multiplication rate of white fluorescent light of 7000 ergs/cm² sec for 20 min every 3 h is reduced from 0.073 to 0.032 by applying near infrared for 30 min after each illumination. In the presence of $3 \cdot 10^{-6} M$ kinetin, the effect of infrared is decreased, but is still important. This partial reversibility by infrared of the stimulation by light means that even the growth-promoting effect of a light intensity as high as 7000 ergs/cm² sec is mainly a photomorphogenetic effect, assuming that photosynthesis is not affected by infrared interruptions.

The stimulating effect of light on growth of *L. minor* also has photomorphogenetic features in its dependency on light intensity. Although in continuous light the optimum intensity is 20,000 ergs/cm² sec for the multiplication rate, and 300 ergs/cm² sec for the frond area, the efficiency of the light decreases sharply above 5 ergs/cm² sec. Below this intensity level the effect of light is much enhanced by kinetin (Figs. 1

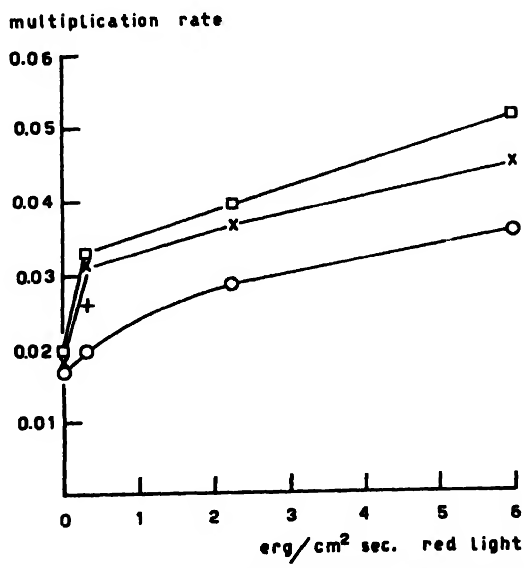

Fig. 1. Light dependency of multiplication rate in *Lemna minor* at different concentrations of kinetin. The medium contained 1% saccharose, Temp. 22°.

$$\text{Multiplication rate} = \frac{\log n_1 - \log n_0}{t_1 - t_0}$$

n_0 and n_1 = number of fronds at times t_0 and t_1. Unit of time = 24 hours. ○ = no kinetin, + = $0.25 \cdot 10^{-6} M$ kinetin, × = $0.5 \cdot 10^{-6} M$ kinetin, □ = $1 \cdot 10^{-6} M$ kinetin.

References p. 380

and 2). Growth in darkness depends on the presence of kinetin in the medium, but also on the light intensity the plants previously received (Fig. 3).

This observation, together with the synergism of kinetin and light as shown in Figs. 1 and 2, indicates that in *L. minor* kinetin cooperates with another substance which accumulates during illumination and enables growth in the presence of kinetin. Since in all experiments the plants had sufficient amounts of sugar, it is not likely that this substance is a primary product of photosynthesis.

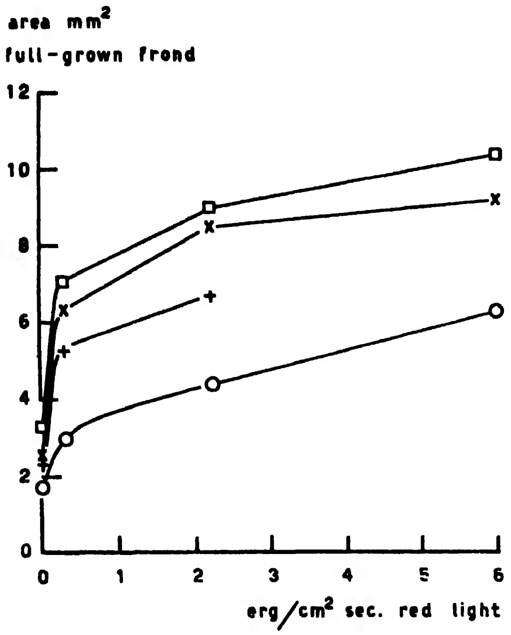

Fig. 2. Light dependency of frond area in *Lemna minor* at different concentrations of kinetin. The medium contained 1% saccharose. Temp. 22°. ○ — no kinetin, + —0.25 · 10⁻⁶ *M* kinetin, × — 0.5 · 10⁻⁶ *M* kinetin, □ = 1 · 10⁻⁶ *M* kinetin.

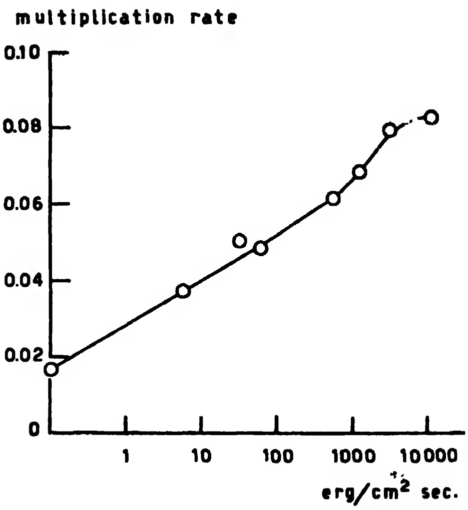

Fig. 3. Multiplication rate of *Lemna minor* during 10 days of darkness. Before darkness the plants received the light intensity indicated on the abscissa for 6 days. The medium contained 2% sucrose and $3 \cdot 10^{-6}$ *M* kinetin. Temp. 22°. Without kinetin, the multiplication rate did not exceed 0.02.

When kinetin is present in the plants in sufficient quantities (*i.e.* $1 \cdot 10^{-6}$ *M* kinetin, Fig. 1), the growth of *L. minor* is controlled by the synthesis of the unknown substance, the concentration of which, in an intensity of 0.3 erg/cm²sec, is already sufficient to increase the growth in the presence of kinetin to 70% of that in dark.

In a prolonged period of darkness there is nearly no growth in the absence of kinetin, even after an illumination period which gives growth if kinetin is present. This suggests that the extra growth without kinetin observable in Figs. 1 and 2 at light intensities above 0.3 erg/cm² sec represents the synthesis of a kinetin-like substance under the influence of light.

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[1] P. R. GORHAM, *Can. J. Research C*, 28 (1950) 356.
[2] W. S. HILLMAN, *Science*, 126 (1957) 165.

* 204th communication.

À propos d'un cas d'ambiphotopériodisme: *Setaria verticillata* Beauv.

INTRODUCTION

Est dite ambiphotopériodique une espèce susceptible de mener rapidement sa mise à fleur en jour long aussi bien qu'en jour court, mais lentement ou pas pour des conditions photopériodiques quotidiennes intermédiaires.

H. Lewis et F. Went[1] ont découvert l'ambiphotopériodisme en 1945 avec *Madia elegans* D. Don., composée annuelle américaine boréo-occidentale. Cette espèce fleurirait en jour long ou continu et en jour court, mais non pas en photopériode quotidienne intermédiaire (12 h).

Le but de ce travail est d'étudier chez une plante ambiphotopériodique les problèmes de morphogénèse et plus particulièrement les rapports entre la croissance et le développement.

MATÉRIEL ET MÉTHODE

La souche de *Madia elegans* que nous avons testée ne s'est pas comportée comme une ambiphotopériodique mais comme une plante de jour long. Mais nous avons découvert dans notre matériel expérimental une espèce présentant des caractéristiques ambiphotopériodiques: *Setaria verticillata* (voir résultats).

Les plantes on été placées dans des cases éclairées soit par des lampes du *type L* (éclairage fluorescent "lumière du jour de luxe" Philips à radiations réparties dans toute la partie visible du spectre. L'éclairement énergétique au sol est de 3,900 ± 1,500 ergs/sec · cm² et à 20 cm du sol de 4,700 ± 1,900 ergs/sec · cm²) soit par des lampes du *type A* (éclairage fluorescent "actinic" riche en ultraviolet de la catégorie A, à maximum vers 3,600–3,700 Å, et émettant dans le visible essentiellement entre 4,000 et 4,400 Å. L'éclairement énergétique au sol est de 2,000 ± 800 ergs/sec · cm² et de 2,600 ± 1,200 ergs/sec · cm²), soit encore par des lampes à incandescence du *type I* (riches en infrarouge à maximum vers 10,000–11,000 Å; dans la partie visible du spectre l'énergie est sensiblement la même que celle du *type L*).

Des expériences ont aussi été faites en éclairement naturel (8 h, 16 h, 24 h — appoint nocturne de type L ou A).

Tous les résultats obtenus — bien que très variables intrinsèquement — ont confirmé l'ambiphotopériodisme de *Setaria verticillata* ainsi que les rapports de dimensions et de fertilité indiqués dans la présente communication pour une série du type L.

RÉSULTATS

Photopériode et vitesse du développement

(1) *Madia elegans*

Nous ne retrouvons pas chez notre souche (Muséum, Paris) de *Madia elegans* l'ambiphotopériodisme des auteurs américains[1]. *Madia* fleurit en éclairement permanent et en jour long mais non pas en jour court et ceci pour les lampes L et I. Nous donnons les résultats de l'expérience lors d'un éclairement du *type L* avec une température variant entre 18° et 25°.

Bibliographie p. 383

Il est probable que, dans notre expérience, les conditions (température, humidité, qualité de la lumière) de milieu ne concordent pas avec celles présentes dans le travail de Lewis et Went ou bien que les souches utilisées soient différentes.

TABLEAU I

Durée de l'éclairement quotidien (h)	*Nombre de jours du semis à la floraison*
24	45
16	65
8	rien après 120 jrs

TABLEAU II

Durée de l'éclairement quotidien (h)	*Nombre de jours du semis à la paniculaison*
24	35
16	70–92
8	28

(2) *Setaria verticillata*

Setaria verticillata Beauv. (= *Panicum verticillatum* L.) est une Graminée annuelle thermocosmopolite caractérisée comme étant une nyctipériodique préférente[2]. Mais les deux souches (souche du Muséum de Paris et souche locale poitevine) que nous avons expérimentées sont apparues comme présentant les caractéristiques de l'ambi-photopériodisme et ceci sous n'importe laquelle des trois lumières utilisées. Nous donnons les résultats de l'expérience lors d'un éclairement du *type L* avec une température variant entre 20° et 23°.

Croissance et développement

Pour des semis de même densité, dans un sol de même qualité, sous éclairement énergétique et température identiques, les plantes cultivées en photopériode quotidienne de 16 h sont toujours plus élevées et leurs panicules sont plus fournies que chez les plantes cultivées en photopériode quotidienne de 8 h. En jour continu, les caractéristiques des plantes sont le plus fréquemment proches de celles des plantes de jour court mais peuvent présenter une grande variabilité. On observe des résultats similaires sous les trois types de lumière à l'exception du fait que dans les conditions du *type L* en 8 h les plantes présentent un rejet fertile plus important que le brin maître. Ce dernier, de plus faibles dimensions, porte un panicule rudimentaire stérile ou à 1 ou 2 caryposes.

Nous donnons les résultats de l'expérience lors d'un éclairement du type L avec une température variant entre 20° et 25°. Chaque variante comporte entre 20 et 25 plantes. Le chiffre entre parenthèses correspond à la moyenne -- calculée sur la taille la plus grande de chaque plante -- lorsqu'il y a plusieurs brins. Les chiffres avant et après la parenthèse correspondent aux extrêmes inférieurs et supérieurs.

TABLEAU III

	Photopériode quotidienne de		
	8 h	*10 h*	*24 h*
Hauteur des plantes en cm	6 (11.4) 15	70 (74.4) 80	10 (17.6) 35
Longueur des panicules en cm	0.3 (0.5) 0.7	5 (6) 7	0.3 (0.9) 7
Nombre de caryopses par panicule	1 (3.5) 5	> 100)	(8.3) 100

DISCUSSION

(1) *Setaria verticillata*, dans nos conditions expérimentales, apparaît comme étant

...e ambiphotopériodique relativement peu exigeante à nyctipériodisme préférentiel.

L'ambiphotopériodisme peut s'interpréter comme la manifestation d'un antagonisme entre deux processus: le processus primaire commun à toutes les plantes (exigence en lumière) et le processus adaptatif des plantes de jour court (entraînant la nécessité d'une nyctopériode).

Lorsqu'une plante ambiphotopériodique vit en jour court, ce sont les processus du type jour court qui régissent la mise à fleur. Lorsqu'elle vit en jour continu, les processus du type jour court sont inhibés par et au profit de processus fondamentaux régis par la lumière. Dans les conditions photopériodiques intermédiaires (16 h), les processus du type jour court sont partiellement inhibés (et peut-être aussi, l'exigence en lumière des processus primaires est-elle partiellement satisfaite). Mais les deux types de processus sont contradictoirement superposés et non complémentaires, sinon le développement ne serait pas freiné.

L'ambiphotopériodisme suggère une interprétation des plantes de jour court comme cas particulier adaptatif des plantes de jour long. Le processus fondamental de mise à fleur serait un processus de jour long, masqué chez les plantes de jour court par un processus adaptatif nyctipériodique devenu nécessaire dans les conditions de vie normales ou para-normales de la plante. Cette interprétation pourrait être vérifiée par l'obtention dans des conditions expérimentales données de manifestations à caractéristiques de type ambiphotopériodique chez les plantes de jour court[4].

(2) La thèse[3] de la modification des rapports entre la croissance et le développement durant le photopériodostade comme facteur essentiel de la modification de la genèse de la structure de l'inflorescence, établie chez les plantes de jour long et chez les plantes de jour court, se trouve vérifiée chez une plante ambiphotopériodique: *Setaria verticillata*.

Pour des conditions de croissance sensiblement identiques (la durée de l'activité photosynthétique est évidemment plus grande en 24 h qu'en 8 h, ce qui — outre le léger retard du développement en 24 h sur 8 h — peut expliquer les plus grandes dimensions de l'appareil végétatif en 24 h par rapport à celles en 8 h), la vitesse d'accomplissement du photopériodostade détermine l'importance de la structure de l'inflorescence: en jour court et en jour continu, le développement est rapide, l'inflorescence est rudimentaire; en conditions photopériodiques intermédiaires, le développement est lent, l'inflorescence est fournie et normalement structurée.

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[1] H. LEWIS AND F. WENT, *Am. J. Botan.*, 32 (1945) 1.
[2] P. CHOUARD, *Bull. soc. botan. France*, 97 (1950) 234.
[3] C. C. MATHON ET M. STROUN, *Cahiers Natural*, 9 (1954) 59; C. C. MATHON ET M. STROUN, *Lumière et floraison, le photopériodisme*, Presses Universitaires de France, 1960; C. C. MATHON, *Bull. soc. botan. France*, 106 (1959) 123; M. STROUN, *Bull. soc. botan. France*, 105 (1958) 1.
M. STROUN *et al.*, in B. CHR. CHRISTENSEN AND B. BUCHMANN (editors), *Progress in Photobiology*, Elsevier, Amsterdam, 1961, p. 384.

Long-day effect as a function of interrelations between light quality, duration of photoperiod, and development phases in *Perilla nankinensis* Voss

INTRODUCTION

Whereas the effect of light quality during flower induction has been widely studied, the problem of the effect on flowering of light quality in the pre- and postinduction phases has been less frequently investigated.

In this paper we shall examine, in plants of *Perilla nankinensis* Voss, (SDP) the "long-day effect"* as a function of interrelations between light quality, the duration of the photoperiod and the development phases (pre-inductive, inductive and post-inductive).

MATERIAL AND METHOD

Our study material consisted of plants of *Perilla nankinensis* Voss obtained from Messrs. Vilmorin.

All experiments were carried out in dark boxes lit with lamps of different wave-lengths. The light sources consisted of 4 luminescent tubes of 40 Watts each, placed in reflectors (Electrical engineering workshops, Charleroi). Relative values for the spectrum distribution curves are given as dotted lines in Fig. 1. for Phytor tubes

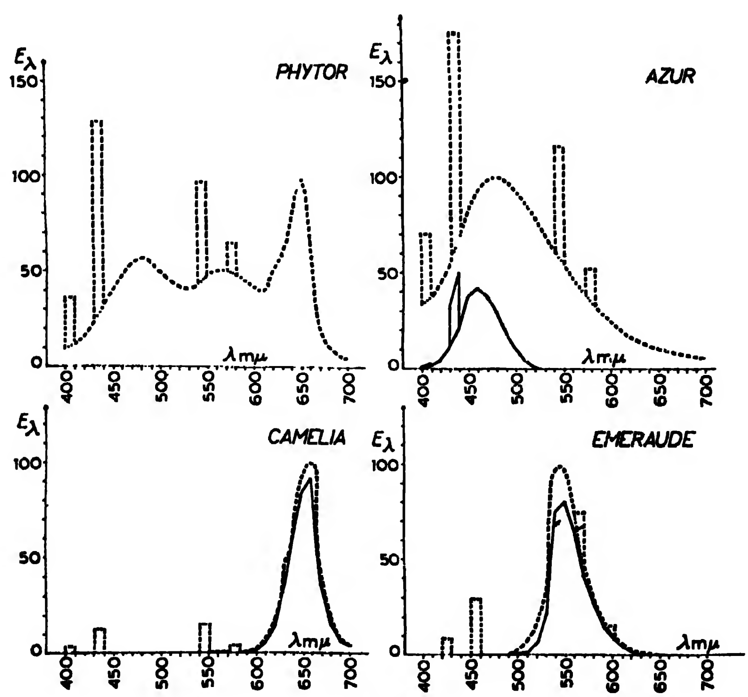

Fig. 1.

* Expression from MEIJER[1] *et al.* meaning: consequence of types of light (duration, quality, intensity) favourable to flower induction in LDP and unfavourable in SDP.

white light), Camelia tubes (red light), Emeraude tubes (green light) and Azur tubes (blue light). Stress must be laid on the fact that all these lamps yield infrared light. In some cases filters were added (Röhm and Haas) to obtain a narrower spectrum distribution curve, shown as a full line in Fig. 1. The energy value of the light in each box varied between 3000 and 4000 microwatts/cm^2 at the level of plant tops[2,3]. The temperature in the boxes was maintained at 26–31°. Immediately after germination the plants were subjected to permanent lighting (pre-inductive phase). As soon as the 5th leaf was out, they were short-day induced (inductive phase); this treatment was applied for a few days, after which they were again subjected to permanent lighting (post-inductive phase). During each phase, the plants in the different series were exposed to the various coloured lights.

RESULTS

The experiment presented here is a confirmation of the results obtained in two similar previous experiments. We draw special attention to these experiments because they were all carried out at the same time.

In the experiment there were four plants in each group. The figures given in Table I are, for the four plants, the average number of days between the end of the inductive

TABLE I

EFFECT OF LIGHT COLOUR DURING PRE- AND POST-INDUCTIVE PHASES ON THE FLOWERING EFFECT OF LIGHT DURING THE INDUCTIVE PHASE

Colour of light used for continuous illumination for 56 days	*Colour of light used for daily illumination for 8 h over 10 days*	*Number of days elapsing between the end of the inductive phase (8 h) and the appearance of shoots in conditions of permanent lighting*			
		white	*red*	*green*	
white	white	20	20	31	25
red	white	20.5	16,5	21	19.5
green	white	14.5	12	11	12
blue	white	18.5	17	26	20.5
white	red	22	21	19	19.5
red	red	18	17.5	21.5	17
green	red	12.5	12.5	13	12
blue	red	19	17.5	30.5	17
white	green	>38	>38	>38	>38
red	green	>38	>38	>38	>38
green	green	13.5	12.5	12.5	13
blue	green	>38	>38	>38	>38
white	blue	25.5	26	>38	21.5
red	blue	25	22	>38	20
green	blue	12	12	12	13.5
blue	blue	26	26	>38	26

period and the appearance of the first shoots. In the case of fractions of days other than 0.5, the nearest whole day was taken.

The following observations may be made on Table I:

(*a*) *During the pre inductive phase* (permanent lighting for 56 days) green light promotes the effect of the ensuing inductive photoperiod, independently of the colour of the light during the inductive or post-inductive phases: G/W/W, G/W/R, G/W/G, G/W/B, G/R/W, G/R/R, G/R/G, G/R/B, G/G/W, G/G/R, G/G/G, G/G/B*. In one of

* The first letter represents the colour during the pre-inductive phase, the second letter the colour during the inductive phase, and the third letter the colour during the post-inductive phase.

our other experiments, a plant flowered after subjection to permanent lighting with green light — the temperature was 21–26°[4].

Under our experimental conditions, red or blue light seemed to be as detrimental to induction as white light.

(*b*) *During the induction phase* (8 h daily for 10 days) red light is as favourable to induction as white light. Under blue-light conditions the first shoots are only slightly delayed but there is a marked delay under conditions of green light: W/G/W, W/G/R, W/G/G, W/G/B, R/G/W, R/G/G, R/G/B, B/G/W, B/G/R, B/G/G, B/G/B. When filters were used (full line in Fig. 1) under blue- or green-light conditions, the plants found it very difficult to produce shoots.

(*c*) *During the post-inductive phase* (permanent light) it is seen that the plants which were blue-light induced and were subsequently placed in permanent green light, produce their shoots rather belatedly: W/B/G, R/B/G, B/B/G.

DISCUSSION

The "long-day effect" of a specific light quality is a function of the duration of the photoperiod. For example, for a "short day", green light has a more pronounced "long day effect" than blue, red or white light. However, for permanent lighting during the pre-inductive phase, white, red or blue light has a more definite "long-day effect" than green light.

The "long-day effect" of a specific light quality is a function of the development phase. For example, green light used for permanent illumination promotes the induction of *Perilla* in the pre-inductive phase and delays it in the post-inductive phase (after blue light induction).

In identical conditions of pre-inductive and inductive phases, the sprouting of shoots is a function of light quality during the post-inductive phase. This tends to confirm the existence, within the stage of photoperiodism, of a sub-stage with specific spectrum requirements, once the flower induction has taken place. For instance, during the post-inductive phase (after blue light induction – minimal or specific conditions?) shoots are induced more quickly by white, blue or red light than by green light.

Further research is necessary to determine the existence of specific relationships between light qualities during the various phases.

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[1] G. MEIJER, *Acta Botan. Neerl.*, 8 (1959) 189.
[2] C.-CH. MATHON AND M. STROUN, *Lumière et Floraison*, Presses Universitaires de France, 1960.
[3] M. STROUN, J. F. SCHOPFER AND F. CHODAT, *Bull. soc. botan. France*, 106 (1959) 309.
[4] G. MEIJER, *Photomorphogenesis in Different Spectral Regions, Intern. Symposium on Photoperiodism and Related Phenomena in Plants and Animals (Gatlinburg, Tennessee, 1957)*. Laboratoria N.V. Philips' Gloeilampenfabrieken Eindhoven, 101, 1952.

Dual effect of red light on the photoperiodic response of *Salvia occidentalis*

In experiments with the SD plant* *Salvia occidentalis* and the LD plant* *Hyoscyamus niger* we found that an interruption in the middle of the long dark period with red nightbreak light only caused a LD effect if the preceding main light period contained sufficient blue or far-red radiation (Table I). From these results it was concluded[1] that two photoreactions are involved in obtaining a long-day effect, *viz.*:

TABLE I

THE INFLUENCE OF THE LIGHT QUALITY OF THE DAILY MAIN LIGHT PERIOD (10 h of light per day) ON THE EFFECT OF RED NIGHTBREAK LIGHT (10 min)

+ generative, − vegetative

main light period (h/day)	*nightbreak (min)*	*Salvia occidentalis (SD plant)*	*Hyoscyamus niger (LD plant)*	*Photoperiodic effect*
10 Red		+	−	SD
10 Red	10′ Red	+		SD
10 Green		+		SD
10 Green	10′ Red	+		SD
10 Green + Far-Red		+		SD
10 Green + Far-Red	10′ Red		+	LD
10 Blue		+	−	SD
10 Blue	10′ Red		+	LD

(*a*) The "nightbreak reaction" regulated by the reversible red, far-red pigment system which is known to control so many phenomena in plant development. (low energy reaction[2])

(*b*) The "main-light-period reaction" which is especially sensitive to blue and far red. This main light period reaction seems to be identical with the "high energy reaction" studied by Mohr[2].

It has been demonstrated that the effectiveness of nightbreak light depends on the moment at which irradiation is started during the dark period. The highest effectiveness is found at about the middle of the dark period. The more the nightbreak is shifted towards the beginning or towards the end of the dark period, the more energy is required[3].

In experiments with *Salvia occidentalis* we found that an irradiation with red light given at the beginning of the long dark period is not only less effective, or even unable to cause a LD effect, but even shows an inhibiting effect on the LD activity of a nightbreak given in the middle of the dark period. As can be seen from Table II, an irradiation with only 15 min of red light was sufficient to prevent the LD effect: 8B–10′R = LD effect, 8B¼R–10′R = SD effect.

* SD = short day, LD = long day.

References p. 388

TABLE II

THE INFLUENCE OF IRRADIATION WITH RED LIGHT FOLLOWING THE MAIN LIGHT PERIOD, ON THE EFFECT OF A NIGHTBREAK TREATMENT

The nightbreak with 10 min of red light was given 8 h after the end of the main light period of 8 h of blue light. + = generative, — = vegetative

Light treatment	*Result*	*Photoperiodic effect*
8B	+	SD
8B – 10′R	—	LD
8B 2R	+	SD
8B 2R – 10′R	+	SD
8B 1R	+	SD
8B 1R – 10′R	+	SD
8B ½R	+	SD
8B ½R – 10′R	+	SD
8B ¼R	+	SD
8B ¼R – 10′R	+	SD

It was then concluded that an LD effect was brought about only when the red, far-red pigment — responsible for the photoperiodic effect — remained in the red-absorbing form during a certain number of hours of the daily cycle (between the main light period and the nightbreak).

However, the story appeared to be more complicated as 10 h of blue light followed by 4 h of red light (10B4R) caused an LD effect and this LD effect was not obtained if nightbreak light was also given (10B4R : LD effect, 10B–15′R : LD effect, 10B4R 15′R : SD effect). Thus, nightbreak light which caused an LD effect after 10 h of blue light (10B–15′R) nullified the LD activity of a supplementary irradiation of 4 h of red light (10B4R). This nullifying effect of nightbreak light could be eliminated either by increasing the length of the nightbreak period to 60 min or by subjection to a further far-red irradiation for 10 min[4].

It appears that the SD plant *Salvia occidentalis* can behave like an LD plant since under certain conditions a nightbreak can cause flowering. A comparable phenomenon has been reported by De Lint[5] for the LD plant *Hyoscyamus niger* which was found to react like an SD plant under certain condition , since flower induction could be inhibited by an interruption of the long dark period.

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1 G. MEIJER, *Acta Botan. Neerl.*, 8 (1959) 189.
2 H. MOHR, *Ergeb. Biol.*, 22 (1960) 67.
3 W. KÖNITZ, *Planta*, 51 (1958) 1.
4 G. MEIJER, *Acta Botan. Neerl.*, 9 (1960) 220.
5 P. J. A. DE LINT, *Nature*, 184 (1959) 731.

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Critical daylength of the short-day plant *Salvia occidentalis* in red and far-red radiation

Salvia occidentalis is a short-day plant which, when grown in white light, flowers only when the daylength is less than 14 h[1]. If grown in light of different colours it is found that green light tends to increase this critical daylength, red light has less influence, and blue light makes the critical daylength a little (not more than 1 h) shorter.

Könitz[2] examined another short-day plant, *Chenopodium amaranticolor*, which has a critical daylength in white light between 14 and 15 h. By interrupting the daily illumination period for 2½ h with far-red radiation the critical daylength could be reduced to 13 h.

The object of the experiment described here was to see whether the critical daylength of *Salvia occidentalis* could be considerably reduced by sufficient red (R) and far-red (FR) irradiation.

The light intensities used were, for the red light $\pm$ 1100 $\mu W/cm^2$ and for the far-red $\pm$ 300 $\mu W/cm^2$. A daily irradiation of 12 h with red light resulted, as would be expected, in flowering (Table I). If, however, this 12 h irradiation was interrupted by 2 h

TABLE I

THE INFLUENCE OF FAR-RED IRRADIATION ON THE PHOTOPERIODIC EFFECT OF A SHORT-DAY TREATMENT OF *Salvia occidentalis*

+ — generative, − — vegetative. Number of plants: 4.

Light treatment (h per day)	*Condition of the growing points*	*Photoperiodic effect*
12R	+ + + +	SD
3R	+ + + +	SD
3R 2FR	+ + + +	SD
3R 2FR 7R	− −	LD
3R 2FR 5D 2R	−	LD
3R 2FR 6R	+ + + +	SD
3R 2FR 4D 2R	− − − −	LD
3R 2FR 5R	+ + + +	SD
3R 2FR 3D 2R	+ + +	SD/LD

of far-red (from the third to the fifth hour) flowering was inhibited. So the sequence 3R 2FR 7R was physiologically a long day to *Salvia occidentalis*. One hour less red light, 3 R 2FR 6R, however, induced flowering. So by this kind of treatment the critical daylength was reduced to a value of 11–12 h. By a slightly different treatment the critical daylength could be made still shorter. A daily sequence 3R 2FR 4D 2R gave only vegetative plants. However 3R 2FR 3D 2R induced flowering.

We were therefore able to reduce the critical daylength for *Salvia occidentalis* from its normal 14 h to 11 h, by exposing the plants to daily cycles of 3 h of red – 2 h of far red – 4 h of darkness – 2 h of red – 13 h of darkness (3R 2FR 4D 2R).

According to Meijer a main-light-period reaction and a nightbreak reaction are necessary. The experiments described in this paper may indicate that after some hours

References p. 390

of red, the far-red irradiation dose may in fact be the main light period. The second period of red irradiation will then act as nightbreak light.

The benificial effect of the dark period after the far red may be explained by the fact that red light given immediately after far-red may antagonize much of the far-red effect.

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[2] W. KÖNITZ, *Planta*, 51 (1958) 1.

Über die Wirkung der Tagesdauer auf das Überwintern von Klee

Auf den Versuchsgütern der Universität Helsinki, in Viik (Südfinnland; 60° 10'N) und in Muddusniemi (Lappland; 69° 5'N), ist im Verlaufe vieler Jahre der Einfluss der Dauer des Sommertages auf die Überwinterung von Klee erforscht worden. Die Dauer der täglichen Lichtperiode ist geregelt worden durch Verdunkelungskästen, die kein Licht, aber doch das Regenwasser durchlassen. Die Temperatur ist innerhalb des Verdunkelungskastens im Mittel dieselbe gewesen wie ausserhalb, wo jedoch der Temperaturwechsel etwas stärker gewesen ist. Die Versuchspflanzen sind in Reihen

Abb. 1. Feldversuch, bei dem der Einfluss der Tagesdauer auf das Überwintern von Rotklee untersucht wurde.

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mit gegenseitigem Abstand von 20 cm gesät worden. Zu einer Probefläche haben sechs Reihen von je 80 cm Länge gehört. Doch hat in Versuchen, in denen die verschiedenen Kleestämme miteinander verglichen worden sind, eine Probefläche nur eine Reihe umfasst (Abb. 1). Es hat 3 Wiederholungen gegeben. Die Aussaat ist Mai-Juni ausgeführt worden, die Saatmenge hat 20 kg/ha ausgemacht. Mit der photoperiodischen Behandlung der Pflanzen ist im allgemeinen im Juni begonnen worden, gleich nach dem Spriessen des Klees, aber in einigen Sonderversuchen auch später, Anfang, Mitte und Ende August; die Behandlung endete am 30. September. Bei den in Südfinnland ausgeführten Versuchen wurde der Bestand schon August-September gemäht; die Stoppellänge betrug 3 cm. Die Überwinterung des Klees wurde in dem auf die Aussaat folgenden Winter untersucht.

Wenn der Winter streng war, überwinterten sowohl Rot- (*Trifoliorum pratense*) als auch Alsikeklee (*T. hybridum*), wenn sie unter Kurztagbedingungen (10 und 13 St.) gewachsen waren, entschieden besser als nach einer Entwicklung unter Langtagverhältnissen (Abb. 2; Tabellen I–VI). In Lappland, wo die Sonne im Sommer ununterbrochen über zwei Monate oberhalb des Horizontes bleibt, ist die Wirkung der Kurz-

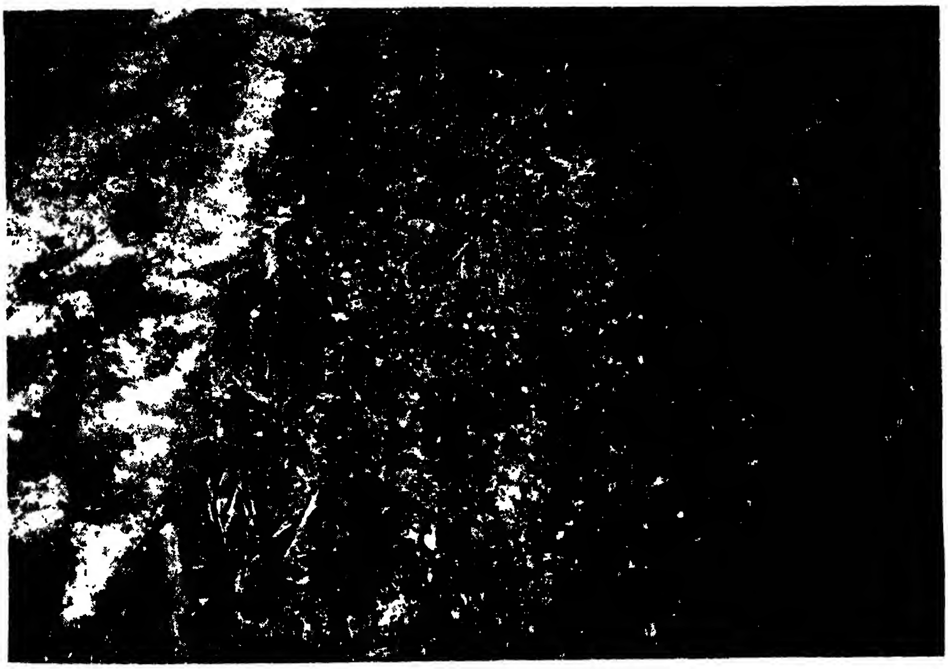

Abb. 2. Das Überwintern von Rotklee unter Kurz- (vorn) und Langtagverhältnissen (hinten).

tagbehandlung auf die Überwinterung des Klees ungefähr dieselbe gewesen wie in Südfinnland, wo der längste Tag nicht ganz 19 Stunden gedauert hat (Tabelle III). Das Kürzen der täglichen Lichtperiode auf 17 Stunden hat bei den in Lapland ausgeführten Versuchen das Überwintern des Klees überhaupt nicht verbessert (Tabelle I). Da mit der Kurztagbehandlung erst Mitte August begonnen worden ist, hat sich die positive Wirkung auf das Überwintern des Rotklees Tammisto in Lappland als nur verhältnismässig gering erwiesen:

Behandlung begonnen	gar nicht	15.8.	22.6.
Überwinterung (0–10):	5.2		9.7

Die Kurztagbehandlung verbesserte das Überwintern aller untersuchten Kleestämme. Das Überwintern finnischer Rotkleestämme (2 Stämme untersucht) verbesserte sie jedoch wesentlich nur in strengen Wintern (Tabelle II). Zu untersuchen waren ausserdem 2 schwedische, 1 österreichischer, 1 schweizerischer, 1 italienischer und 14

TABELLE I

DIE WIRKUNG DER TAGESDAUER AUF DAS ÜBERWINTERN DES ROTKLEES TAMMISTO (FINNLAND) UND DES ALSIKEKLEES TAMMISTO (FINNLAND) IN LAPPLAND 1949 50 (0 10; 10 = völlig am Leben, 0 = abgestorben)

Versuchspflanze	*Überwinterung (0–10)*		
	Norm. Tag	*17 Std.*	*10 Std.*
Rotklee	2.3	1.8	8.0
Alsikeklee	0.8	1.5	6.0

TABELLE II

DIE WIRKUNG DER TAGESDAUER AUF DAS ÜBERWINTERN DES ROTKLEES TAMMISTO

Versuchsstelle	*Winter*	*Überwinterung (0–10)*	
		Norm. Tag	*10 Std.*
Südfinnland	1947 -48a	0.5	9.8
Südfinnland	1947 48b	6.0	10.0
Südfinnland	1956 57	8.6	9.8
Südfinnland	1958–59	9.4	9.0
Südfinnland	1959 60	9.2	10.0
Lappland	1949–50	2.3	8.0
Lappland	1958 59	8.5	9.7
Lappland	1959 -60	9.8	10.0

TABELLE III

DIE WIRKUNG DER TAGESDAUER AUF DAS ÜBERWINTERN VON 21 ROTKLEESTÄMMEN 1958 59 AUSSAAT AM 23.5.1958

Überwinterung nach Normaltag-dauer 0–10	*Südfinnland*			*Lappland*		
	Anzahl der Klee-stämme	*Überwint. (0–10) Normaltag*	*10 Std.*	*Anzahl der Klee-stämme*	*Überwint. (0-10) Normaltag*	*10 St.*
über 8	2	8.8	9.1	4	9.1	8.9
8–5	6	5.4	9.2	1	6.3	8.7
5–2	6	3.7	7.1	8	3.4	8.5
unter 2	7	0.9	2.5	8	0.5	5.3

TABELLE IV

DER EINFLUSS DER TAGESDAUER AUF DIE GESCHWINDIGKEIT DER ENTWICKLUNG UND DAS ÜBER- 'ON 21 ROTKLEESTÄMMEN IN SÜDFINNLAND 1958–59. AUSGESAT

*Entwicklungsstufe am 4.8.1958**	*Anzahl der Klee-stämme*	*Überwinterung (0–10)*	
		Normaltag	*10 Std.*
Rosette	5	6.4	9.5
Luftsprosse	9	3.9	6.9
Knospen	6	1.6	4.0
Blühend	1	0.0	0.1

* Normaltag; bei 10-stündiger Tagesdauer blieben alle Kleestämme bis Winteranfang im Rosettenstadium.

TABELLE V

DIE WIRKUNG DER TAGESDAUER AUF LUFTSPROSSENBILDUNG UND ÜBERWINTERUNG EINIGER ROTKLEESTÄMME IN SÜDFINNLAND 1959–1960. AUSGESÄT AM 24.4.1959

Kleestamm	*Anzahl der Luftsprosse je Reihenmeter am 14.8.1959*			*Überwinterung (0–10)*		
	Normaltag	*13 Std*	*10 Std.*	*Normaltag*	*13 Std.*	*10 Std.*
Kusträsk, Schweden	1	0	0	9.5	9.7	9.8
Tetra Jo TPA 1, Finnland	5	0	0	9.7	10.0	10.0
Tammisto, Finnland	9	0	0	9.2	10.0	10.0
Viik, Finnland	15	0	0	9.8	10.0	10.0
Reichersberger, Österreich	49	0	0	8.7	10.0	10.0
Idaho x 59569, USA	69	5	0	7.0	10.0	10.0

TABELLE VI

DIE WIRKUNG DER TAGESDAUER AUF DAS ÜBERWINTERN EINIGER ROTKLEESTÄMME IN LAPPLAND 1959–60

Kleestamm	*Überwinterung (0–10)*		
	Normaltag	*13 Std.*	*10 Std.*
Tammisto, Finnland	8.9	10.0	10.0
Viik, Finnland	9.5	10.0	10.0
Tetra Jo TPA 1, Finnland	6.2	9.9	10.0
Kusträsk, Schweden	9.4	10.0	10.0
Essi, Schweden	0.1	9.3	10.0
Mammoth x 49305, USA	0.0	9.3	10.0

USA-Rotkleestämme. Mit Ausnahme des schwedischen Kusträsk- und des amerikanischen Alaskland F.C.24316-Rotkleestammes überwinterten die ausländischen Rotkleestämme bei Langtagverhältnissen auch dann mangelhaft, wenn der Winter der Kleeüberwinterung verhältnismässig günstig war (z.B. Tabelle V und VI). Kurztagbehandlung verbesserte dann durchaus das Überwintern dieser Stämme. Wenn der Winter streng war, starb auch der unter Kurztagbedingungen gewachsene Klee ab. Zugleich stellte sich heraus, dass diejenigen Kleestämme, die, unter Langtagverhältnissen gewachsen, am allerschlechtesten überwinterten, auch bei Kurztagbehandlung den Winter schlechter als die übrigen überstanden (Tabelle III). Diejenigen Eigenschaften des Klees, die sein Verhalten zur Tagesdauer bestimmen, erwiesen sich also mit Rücksicht auf die Überwinterung des Klees als wichtig; zugleich aber stellte sich heraus, dass auch die übrigen Eigenschaften des Klees in dieser Hinsicht von beträchtlicher Bedeutung sind.

Bei den in Lappland ausgeführten Versuchen war die Entwicklung des Klees, offenbar infolge der niedrigen Temperatur, so langsam, dass kein einziger der untersuchten Rotkleestämme im Aussaatsommer dazu kam, sich über das Rosettenstadium hinaus zu entwickeln. In Südfinnland dagegen bildete der Rotklee schon im ersten Sommer Luftsprosse aus, ja sogar Knospen und Blüten. Zugleich war festzustellen, dass die Rotkleestämme im allgemeinen um so winterhärter waren, je langsamer sich ihre reproduktive Entwicklung gestaltete (Tabelle IV). Diejenigen Rotkleestämme, die sich unter Langtagbedingungen am schnellsten entwickelten und in dem darauffolgenden Winter am schlechtesten überwinterten, überstanden auch bei Heranwachsen unter den bei täglich 10-stündiger Lichtdauer bestehenden Verhältnissen den Winter am schlechtesten, obgleich auch sie dann bis Winterbeginn im Rosettenstadium

blieben (Tabelle IV). Es scheint also, dass die Geschwindigkeit der reproduktiven Entwicklung des Rotklees dennoch nicht einzig seine Winterfestigkeit entscheidet. Bei einem im J. 1959 angelegten Versuch bildete der Klee Idaho x 59569 in gewissem Masse Luftsprosse auch dann aus, wenn er unter den Verhältnissen einer täglich 13-stündigen Lichtdauer gewachsen war; trotzdem überwinterte er vollkommen (Tabelle V).

Bei den obenbeschriebenen Versuchen sind weder Kleekrebs (*Sclerotinia trifoliorum*) noch sonstige Auswinterungspilze nennenswert vorgekommen. Bei einem Versuch (Tabelle II; Winter 1947–48a) war das Versuchsfeld im Winter verhältnismässig lange Zeit von oberflächlichem Eis bedeckt. Im Winter 1959 60 ist derartiges Eis nicht aufgetreten; nach dem Abschmelzen des Schnees sind ebensowenig den Klee schädigende Fröste vorgekommen; ausserdem ist die Temperatur unter dem Schnee nicht sehr niedrig gewesen: in Südfinnland —2.8 bis + 1.0°, in Lappland —4.7 bis + 1.0°. Doch gingen einige Kleestämme bei Heranwachsen unter Langtagverhältnissen recht beträchtlich ein; dagegen überwinterten sie bei Kurztagbehandlung nahezu vollständig (Tabelle V und VI). Als ununterbrochen während des Winters unter dem Schnee Kleeproben entnommen wurden, konnte festgestellt werden, dass die Pflanzen der empfindlichsten Kleestämme allmählich schwächer wurden. Nachdem sie herein gebracht worden waren, wuchsen sie im Vorfrühling kümmernd, und ein Teil der Individuen erwies sich schon vor der Schneeschmelze als abgestorben.

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Failure of reversibility of the photoreaction controlling plant growth

This report deals briefly with the expression and failure of photoreversibility of flowering in seedlings of Japanese morning-glory, *Pharbitis nil* Chois. (*Ipomoea nil* (L) Roth)[1], which is in marked contrast to the photoreversibility of flowering response in *Xanthium* sp. and *Soja max* sp. Nakayama[2,3] and Takimoto and Ikeda[4] studied photoperiodic responses of *Pharbitis* including red, far-red reversibility and Nakayama reviewed earlier literature.

The plants used were from seeds brought from Japan by Nakayama and were in some cases from a strain that required only one inductive cycle for flowering and in others from one that required two or more cycles. The cultural methods were essentially those described by Nakayama[3]. For action-spectrum experiments one cotyledon was removed from each seedling and the seedlings were irradiated in the spectrum of the spectrograph described by Parker *et al.*[5].

Action-spectra were measured at the beginning (Figs. 1 and 2) and at the middle (Fig. 3) of the dark period. For those at the beginning of the dark period the plants were either placed directly in the spectrum at the end of the photoperiod (Fig. 1) or were first given an inhibitory irradiation at an energy of about 120 mJ in the wavelength region from 700 to 800 mμ from a filtered source. They were then placed in the spectrum (Fig. 2). In the former instance flowering was sharply inhibited at wave-

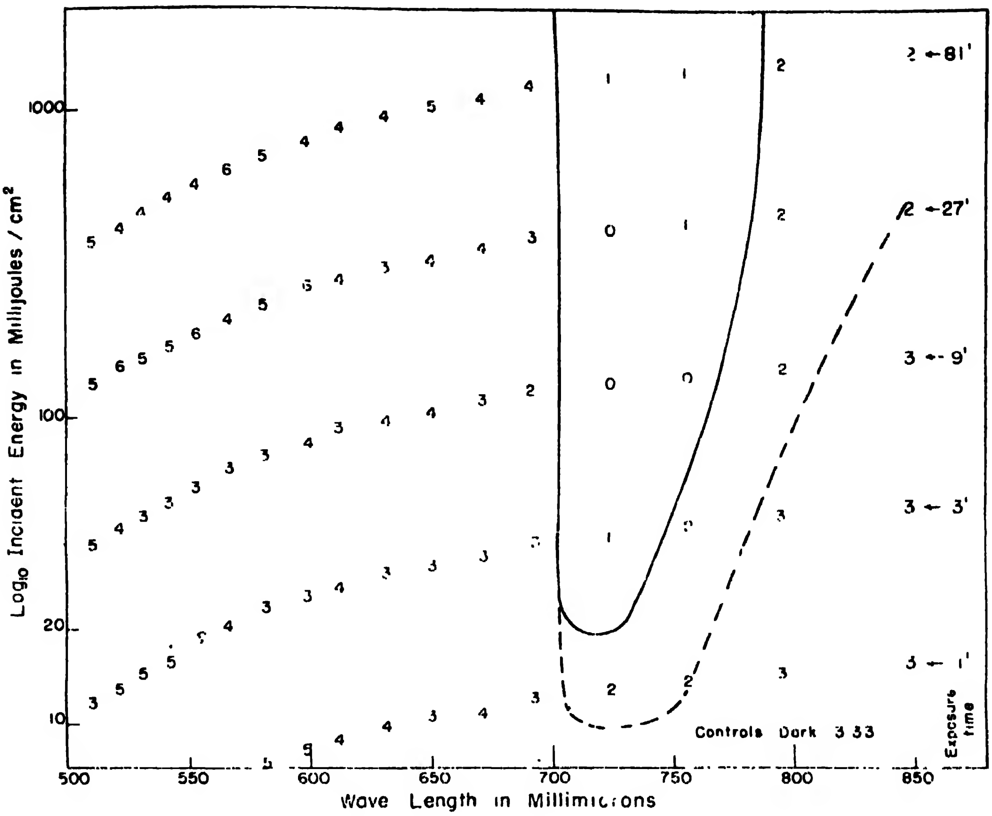

Fig. 1. Action spectrum for floral initiation in seedlings of irradiated *Pharbitis nil* at the beginning of each of three successive daily dark periods. Numbers on this and succeeding figures indicate number of flower buds per plant at dissection. The solid and broken curves indicate two levels of inhibition

lengths > 700 mμ and was uninfluenced or possibly slightly enhanced at wavelengths < 700 mμ. This effect was unchanged throughout the approximately 100-fold range of energies tested.

A similar final result was obtained (Fig. 2) when seedlings were given an initial far-red irradiance that potentially inhibited flowering from the level of six buds per plant in the dark controls to two buds per plant of the far-red controls. Flowering remained at the level of the far-red controls for all lots that received wavelengths > 700 mμ regardless of energy. Reinduction of flowering at wavelengths between 600 and 700 mμ was complete at the lowest energy tested and improved progressively at shorter wavelengths with increasing energy.

Flowering of seedlings placed in the spectrum in the middle of the night was inhibited only in the region of 660 mμ at the lowest energies tested (Fig. 3). As energy increased inhibition occurred at both longer and shorter wavelength stations until at the highest energy flowering was inhibited at all but the two shortest wavelength stations.

Red radiant energy was promotive of flowering during the first 2 hours after the beginning of the dark period but became inhibitory about 6 hours later. At the beginning of the dark period far-red reversed the action of red, but near the middle of the dark period it did not.

References p. 398

Reversibility of flowering in *Pharbitis* seedlings was lost between the beginning and the middle of the 16-h dark period and was evidently regained during the ensuing 8-h photoperiod. In one experiment high-intensity photoperiods of less than 8 h were tested to find the duration required to reinduce flowering and that for the reappear-

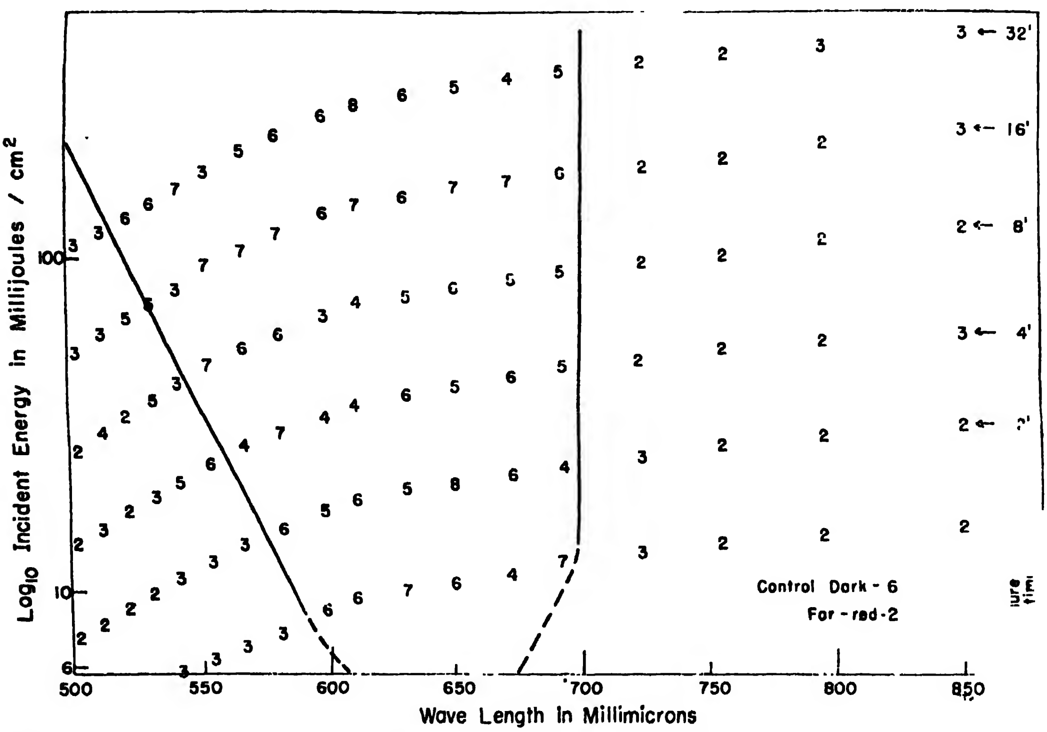

Fig. 2. Action spectrum for floral initiation in seedlings of *Pharbitis nil* in which flowering was potentially inhibited by an irradiance of 240 mJ/cm² from a filtered source in the region of approximately 700 to 800 mμ 16-h dark periods. The seedlings were then immediately irradiated in the spectrum. Reinduction of flowering is indicated at wavelength stations between the solid lines.

ance of reversibility. Plants of the one-induction strain given 8 h of darkness were tested for reversibility either immediately or after 1/4-, 1/2-, 1-, 2-, or 4-h light periods of about 2,000 fc. Only lots that received 2 or 4 h of light flowered and both of these were reversible.

DISCUSSION

The photoreaction controlling flowering may be written as

$$P_{660} \underset{730\ \mathrm{m}\mu\ \max}{\overset{660\ \mathrm{m}\mu\ \max}{\rightleftharpoons}} P_{730}$$

in which P_{660} and P_{730} indicate the red- and far-red-absorbing forms, respectively, of the photoreversible pigment, phytochrome. The minimum energies at 660 and 730 mμ to effect reversal of flowering at the beginning of the dark period and at 660 mμ to inhibit flowering at the middle of the dark period were about 6 mJ/cm² and approximately equal. A far-red irradiance after red in the middle of the dark period did not

reinduce flowering and when applied without a previous red irradiance was inhibitory at energies > 200 mJ/cm².

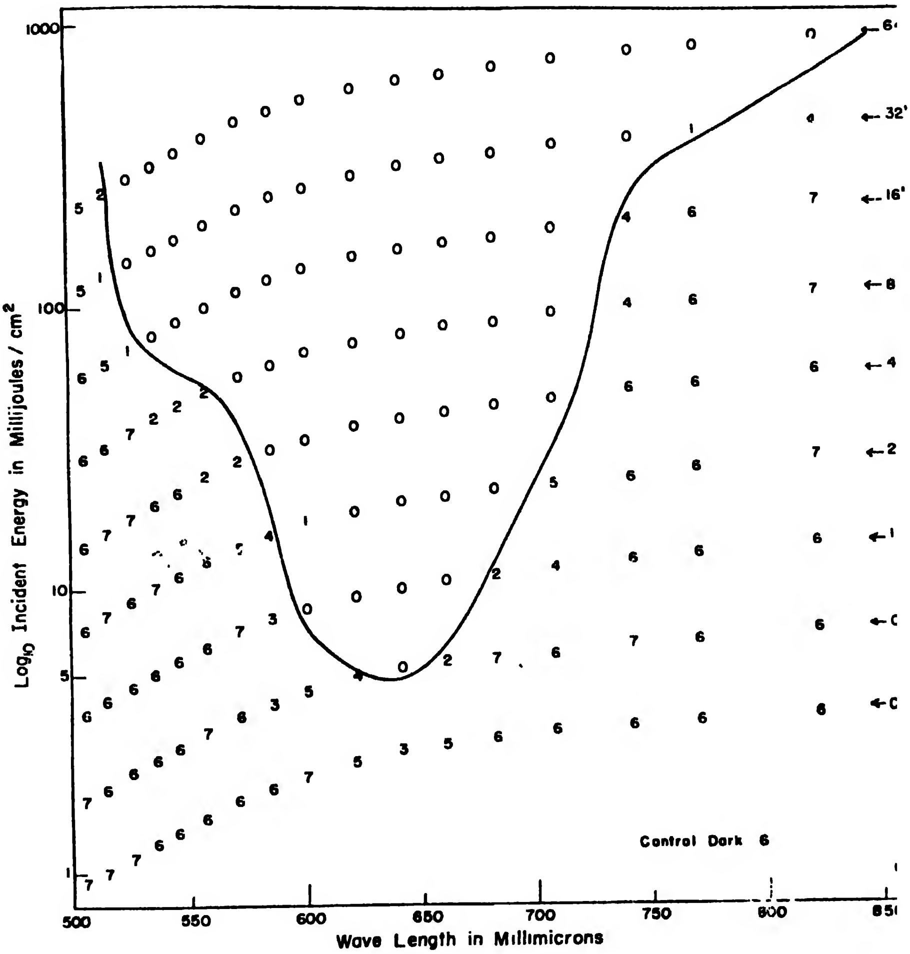

Fig. 3. Action spectrum for floral initiation in seedlings of *Pharbitis nil* irradiated in the middle of each of three successive 16-h dark periods. Inhibition of flowering is shown above the curve.

These experiments indicate that the phytochrome pigment was predominantly in the form P_{730} at the close of the photoperiod and was alternately convertible to P_{660} and P_{730} by successive irradiances of red and far-red. At the middle of the dark period the pigment was effectively in the P_{660} form and was converted to P_{730}, the flower-inhibiting form, by a red irradiance. A subsequent far-red irradiance, however, did not reconvert P_{730} to P_{660}. In the spectral region > 700 mμ, where the wing of the absorption curve of P_{660} lies under the absorption curve of P_{730}, the only action indicated was a weak absorption by P_{660} (Fig. 3).

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References p. 398

[1] S. NAKAYAMA, H. A. BORTHWICK AND S. B. HENDRICKS, *Botan. Gaz.*, 121 (1960) 237.
[2] S. NAKAYAMA, *Ecol. Rev.*, 14 (1958) 325.
[3] S. NAKAYAMA, *Sci. Repts. Tôhoku Imp. Univ.*, 24 (1958) 137.
[4] A. TAKIMOTO AND K. IKEDA, *Botan. Mag. Tokyo*, 72 (1959) 181.
[5] M. W. PARKER, S. B. HENDRICKS, H. A. BORTHWICK AND N. J. SCULLY, *Botan. Gaz.*, 108 (1946) 108.

The promotion of photosensitive growth by sugars and cobalt in etiolated pea stem sections

Light promotes many aspects of de-etiolation in higher plants: leaf expansion, opening of hypocotyl hooks, development of vascular tissue, formation of protochlorophyll, and rate of stem growth. The red, far-red reversible photoinhibition of growth in excised stem sections affords a situation where it is possible to determine whether the inhibitory action of light is specific for growth promotions induced by various factors. If the action of light is relatively specific, then it might be possible to delineate the particular growth system or systems which are controlled by the red, far-red reversible photoreaction.

The experimental procedures have been described in detail earlier[1]. Excised pieces of immature, dark-grown stem tissue were floated on standard buffer (0.02 M PO_4, KH_2PO_4-Na_2HPO_4, pH 6.0), irradiated with red light from 4 red fluorescent tubes (20 min, 1000 erg/cm^2/sec), subsequently incubated in various media for 20 h or more in darkness (26°–28°), after which growth in length and fresh weight was measured. Similar results were obtained for various incubation periods (20–70 h), as well as for elongation and fresh weight measurements. Manipulations were carried out in the dark, with the use of a dim green safe light. All experiments utilized 5 mm sections of apical tissue harvested from the third internode, just below the apical hook, of 7–8 day old dark-grown Alaska pea seedlings.

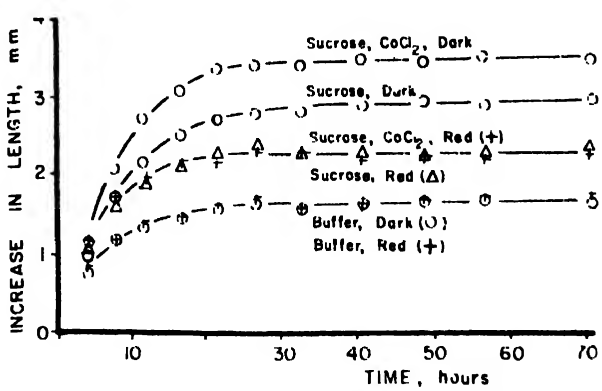

Fig. 1. Time-course of the photoinhibition of stem section growth. Length of sections in the incubation medium was measured at intervals under a dim green safe light. Concentrations: 2% sucrose; $2 \cdot 10^{-5}$ M $CoCl_2$. Each point represents the mean of 12 sections.

Fig. 1 shows the effect of red light on the time course of growth of excised tissue incubated in buffer alone, in 2% sucrose, and in 2% sucrose plus $2 \cdot 10^{-5}$ M $CoCl_2$.

No other substance was included in the incubation media. Light was ineffective in inhibiting growth in basal buffer medium, but did inhibit growth in those sections which were promoted by inclusion of sucrose in the medium. Addition of cobalt to the sucrose-containing medium induced an additional growth promotion in darkness, and this growth was also inhibited by the brief exposure to red light. The effects of red light shown in Fig. 1 are completely reversible by subsequent irradiation with far-red light.

The red irradiation given in Fig. 1 was a saturating dose. Sections incubated in sucrose were inhibited by this dose to the same level as sections incubated in medium containing both sucrose and cobalt. This photoinhibition of growth to the same level occurs in all experiments concerned with sucrose and cobalt. However, the level of photoinhibition does not always include all of the growth induced by sucrose, or sucrose and cobaltous ion.

If indole-3-acetic or gibberellic acids are added to the incubation medium, they stimulate growth, but this growth is insensitive to light. In the presence of sucrose and one of these growth substances, the photoinhibition is never larger than that occurring with sucrose alone. At super-optimal concentrations of growth substances, the photoinhibition in the presence of sucrose may be reduced or eliminated.

The optimum cobalt concentration for growth promotion was found to be about 2-5 · 10^{-5} M. Fig. 2 shows the effect of light on growth of tissue incubated in the presence of the divalent ion of Mn, Mg, Co, and Ni, at a concentration of 2 · 10^{-5} M. $MnCl_2$ and $MgSO_4$ did not affect growth or photoinhibition in medium containing sucrose. These two salts have been tested for growth promoting activity at concentrations between 10^{-6} and 10^{-2} M, but the only effect obtained in the present experimental system was growth inhibition at the higher concentrations. Cobalt induced photosensitive growth regardless of the particular salt utilized; the anions chloride, sulfate, and nitrate having no effect on growth at this low concentration.

Fig. 2 shows that nickel promoted growth to the same level as cobalt (the two ions have the same optimal concentration in this system), but unlike cobalt, nickel induced

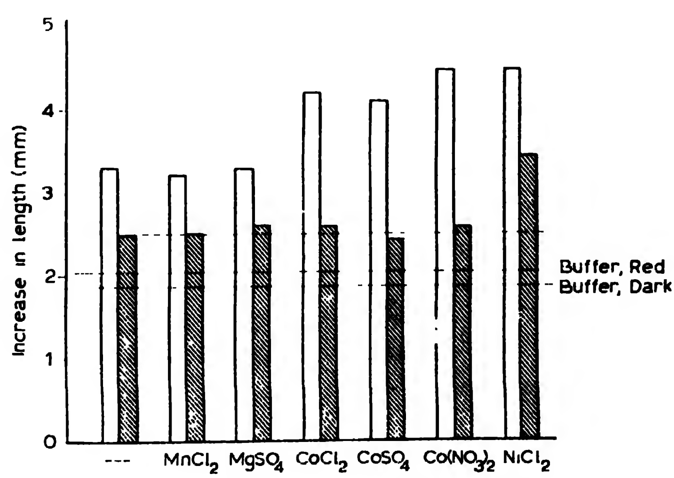

Fig. 2. Effect of light on growth induced in the presence of various metal salts. All salts at a concentration of 2 · 10^{-5} M, in the presence of 1% sucrose. Incubation period 50 h. White bars indicate growth in darkness, shaded bars indicate growth after exposure to red light. Each bar represents the mean of 12 sections.

References p. 400

growth which was not inhibited by light. That is, the photoinhibition of sections promoted by inclusion of nickel in the incubation medium was not significantly larger than the photoinhibition of sections incubated in sucrose alone. Growth induced by nickel has been found to be insensitive to light regardless of nickel concentration or light dose.

The effect of cobaltous ion in inducing photosensitive growth seems to be specific for that ion, although a number of growth-promoting sugars induce photosensitive growth (glucose, fructose, mannose, ribose, xylose). Although cobalt may not be available in sufficient concentrations in intact seedlings to be a normal factor in etiolation phenomena, the ion does seem to affect some system which is closely related to etiolation phenomena. Both cobalt and light promote expansion of etiolated leaf discs[2] and opening of etiolated plumular hooks[3]. Miller[2] noted that etiolated plant tissues which were promoted by cobalt were also acted upon by light. However, the precise relation between cobalt and red light in etiolation is not clear, since cobalt always promotes growth of etiolated tissue, but light may either promote or inhibit growth. Miller[2] postulated that cobalt and light reduce the same growth-limiting condition in leaf disc expansion, although they might reduce it by different pathways. This hypothesis is consistent with photoinhibition of a sugar-cobalt growth system in stems, if the limiting condition may be either removed or introduced by red light depending on the particular tissue involved. Such a complex action of light is not unlikely considering the diversity of effects involved in photomorphogenic changes in patterns of seedling growth.

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[1] W. F. BERTSCH AND W. S. HILLMAN, *Am. J. Bot.*, (1961) in the press.
[2] C. O. MILLER, *Plant Physiol.*, 27 (1952) 408.
[3] W. H. KLEIN, in R. B. WITHROW (Editor), *Photoperiodism and Related Phenomena in Plants and Animals*, Am. Ass. Advance. Sci., Washington D. C., 1959.

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Influence on root growth of light, iron and gibberellic acid

Ordinary roots of higher plants etiolate in darkness and their growth is arrested by visible light. This has been studied on excised wheat roots cultured in nutrient solutions. The inhibition depends partly upon a reduction in cell multiplication in the meristem, partly upon a reduced rate of elongation of the cells. Only this latter effect will be dealt with in the following. The experimental technique employed[1] allows the rate of cell elongation in a restricted sense to be separated from both cell multiplication and duration of cell elongation, which is essential for the interpretation of the light inhibition. This occurs only if the roots are supplied with Fe (10^{-6} M). In the absence of external Fe the roots are practically light-insensitive. Gibberellic acid (10^{-6} M) increases the rate of cell elongation in the light, but has no such effect in darkness. Superficially the inhibition by Fe + light is reversed by GA. The roots also form chlorophyll in light, in the innermost cell layer of the cortex beginning at the end of the zone of elongation. It is increased by Fe and decreased by GA. There is an inverse linear relation between rate of elongation and amount of chlorophyll formed. The results suggest some connexion between inhibition of elongation and formation of chlorophyll, and a direct action of at least Fe on the chlorophyll formation. The experimental data have been published elsewhere[1].

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[1] H. BURSTRÖM, *Physiol. Plantarum*, 13 (1960) 597.

On the hormonal mechanism of photoinhibition of plant stem growth

Growth and form of plants are regulated by both intensity and spectral distribution of incident visible radiation. It has been learned that control of stem growth by visible radiation is mediated through the plant growth hormone gibberellin. Other morphogenic responses to light, *e.g.*, leaf expansion, rate of node formation, etc., are completely independent of gibberellin.

It is known that light inhibition of stem growth of many dark-grown plant species can be quantitatively reversed by gibberellin treatment. The only reasonable kinetic interpretation of the reported results is that light affects the level of endogenous gibberellin[1] (a kinetic analysis of growth factor interactions is being prepared), presumably either by interfering with gibberellin synthesis or diverting gibberellin to some other process. It is interesting to note that examples of the Cucurbitaceae, in which photoinhibition is not reversed by applied gibberellin A_3, show complete reversal of inhibition when treated with gibberellin A_4[2]. More recently it has been found that the red-far-red influence on stem growth of greenhouse-grown Pinto beans

References p. 402

(*Phaseolus vulgaris L.*), and other plants[3] can also be prevented by saturating gibberellin treatments.

Pinto beans have proved to be extremely sensitive to light intensity in addition to their sensitivity to the red/far-red spectral ratio. Plants grown in 50% shade elongate 2–3 times more rapidly than plants grown under full greenhouse solar radiation (*ca.* 80,000 Lux). Further reduction in light intensity results in gradually decreasing growth rates, due to decreasing photosynthesis. On the other hand, the decrease in growth rate as a result of high light intensities is, apparently, the result of a decrease in natural gibberellin at these high intensities. This is demonstrated by the observation that when plants are saturated with gibberellin (by external treatment) they grow most rapidly in full sun. At 50% shade, growth is nearly as great as in full sun, while further decreases in light intensity result in marked decreases in growth — since photosynthesis becomes a concurrent "limiting factor" at low light intensities.

At the present time it appears that two classes of plants may be distinguished. The first class is that represented by the Pinto bean, in which stem growth is almost completely limited by the level of endogenous gibberellin, demonstrated by the extreme response to gibberellin treatments. Such plants show marked increases in stem growth as a result of partial shading. The other class is represented by Alaska pea, which shows little response to applied gibberellin, and only slight increases in growth at reduced light intensities. Thus, some plants, by their genetic nature, are nearly saturated with endogenous gibberellin under good growing conditions. Increasing the level of gibberellin by lowering the light intensity has little influence on growth rate in this type.

Much further work is necessary to determine whether this may be considered a general physiological distinction between different plant types.

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[1] J. A. LOCKHART, *Plant Physiol.*, 34 (1959) 457.
[2] J. A. LOCKHART AND P. H. DEAL, *Naturwissenschaften*, 47 (1960) 141.
[3] R. J. DOWNS, S. B. HENDRICKS AND H. A. BORTHWICK, *Botan. Gaz.*, 118 (1957) 119.

Remarques sur la portée des différences quantitatives entre les exigences des plantes de jours longs et celles des plantes de jours courts vis-à-vis des éclairements réduisant ou interrompant la durée de la nuit longue

RÉSUMÉ

On sait que la réduction de la durée de la nuit longue par un "*appoint*" d'heures de lumière blanche ou colorée permettant de dépasser alors en éclairement la durée minimale de la photopériode critique, aussi bien que "*l'interruption*" de la nuit longue en son milieu par un court éclairement blanc ou rouge clair, exercent, l'une comme l'autre, un "effet de jour long" sur les deux principales sortes de plantes sensibles au photopériodisme. Un éclairement court en rouge sombre (infrarouge) immédiatement

consécutif à l'interruption en rouge clair ou en blanc, annule, en général, l'effet de jour long qui aurait été donné par ce dernier éclairement seul.

Cependant, ces résultats présentent de nombreuses variantes d'espèce à espèce, mais surtout ils montrent une grande différence quantitative *généralement* observée entre l'ensemble des plantes de jours courts d'une part, et l'ensemble des plantes de jours longs d'autre part. La portée de cette différence ne semble pas assez soulignée.

Chez les plantes de jours courts, l'effet de jour long est déjà obtenu, dans les deux procédés expérimentaux rappelés ci-dessus, avec des éclairements faibles, de l'ordre de quelques lux (en blanc), souvent moins de 10 à 30 lux, pendant la durée suffisante. Chez les plantes de jours longs, il en faut souvent 5 à 10 fois plus, parfois 100 fois plus, et cet appoint n'a son efficacité que si l'éclairement fondamental court qui précède (que j'appelle éclairement trophique) a été suffisamment intense.

L'interruption de la nuit longue par le bleu ou le rouge clair provoquant un effet de jour long, l'annulation de cet effet par le rouge sombre consécutif (quand cette annulation se produit) sont obtenues chez les plantes de jour court par des énergies lumineuses de l'ordre de 100 à 300 ergs/cm^2/sec pendant des durées d'environ 15 sec à 2 ou 3 min. Mais chez les plantes de jours longs, il faut en général des énergies de l'ordre de 10 à 100 fois plus grandes, soit par l'intensité, soit par la durée; parfois le minimum de l'intensité efficace approche de l'intensité de la lumière solaire naturelle, du moins chez certaines espèces; parfois même les courtes interruptions les plus intenses demeurent inefficaces pour provoquer l'effet de jour long.

La détermination quantitative des durées et des intensités efficaces relativement à de telles différences, ne semble pas avoir fait l'objet d'un nombre suffisant d'observations bien contrôlées. Je voulais seulement attirer l'attention sur cette lacune partielle dans nos connaissances, telle qu'elle ressort d'un grand nombre d'essais préliminaires dans mon laboratoire. En effet, tout se passe comme si les plantes de jours courts subissaient, notamment par l'interruption de la nuit longue, une sorte de "déclic" libérant une réaction exothermique qui ramène à néant les préliminaires de l'induction florale, tandis que chez les plantes de jours longs il faudrait une élaboration de substances dépendant de l'énergie lumineuse reçue et de sa longueur d'onde pour achever de conduire l'induction florale à son terme.

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Quelques effets d'éclairements colorés sur *Chenopodium polyspermum*

Chenopodium polyspermum L., plante de jour court préférante[1], fleurit à un stade de développement très précoce sous 8 h d'éclairement: les cotylédons s'épanouissent, les deux premières feuilles s'ébauchent et l'apex devient florifère (il donne une fleur, rarement deux) (Fig. 1).

Conditions expérimentales

Les éclairements colorés sont fournis par des tubes fluorescents dont les radiations sont sélectionnées par des filtres colorés de plexiglas. L'infra-rouge proche est obtenu

Bibliographie p. 406

par l'emploi de lampes à incandescence sous-voltées et de deux filtres rouge et bleu. Ces éclairements colorés sont de l'ordre de 200 erg/cm²/sec. L'éclairement blanc, de l'ordre de 2,000 erg/cm²/sec, est produit par des tubes fluorescents "Blanc Soleil de Luxe".

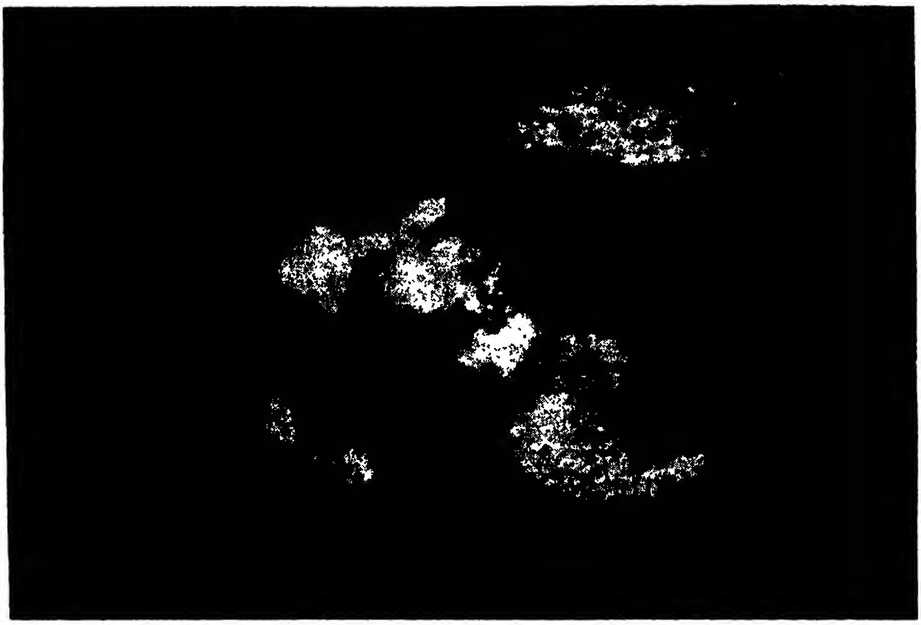

Fig. 1. Plante florifère montrant: une fleur, deux jeunes feuilles, deux cotylédons.

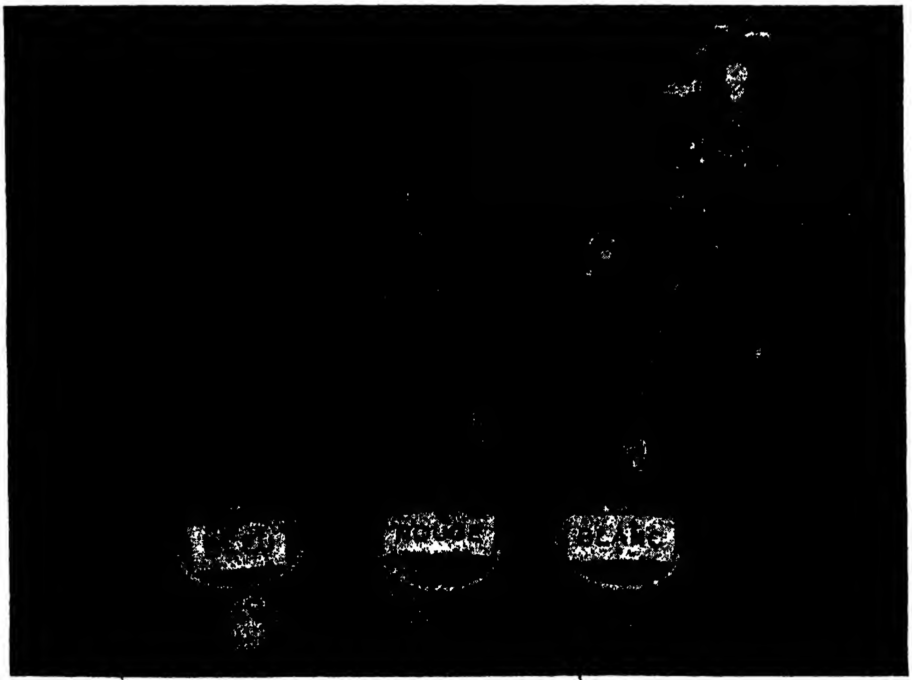

Fig. 2. Action des éclairements colorés d'appoint de 15 h sur la floraison.

Résultats expérimentaux

Dans une première série d'expériences les plantes reçoivent un éclairement blanc de base de 9 h suivi d'un éclairement coloré de 15 h. Contrairement au comportement de *Salvia occidentalis* étudiée par Meijer[3], la floraison de *Chenopodium polyspermum* est beaucoup plus rapide dans le bleu que dans le rouge et le blanc (Fig. 2). La croissance végétative se déroule comme chez les espèces étudiées par Wassink et Stolwijk[5,6], c'est à dire qu'elle est plus active (nombre et taille des feuilles, présence de rameaux secondaires) dans le rouge puis dans le blanc et en fin dans le bleu.

Fig. 3. Effet des éclairements rouge et infra-rouge interrompant la nuit longue.

Dans une deuxième série d'expériences les éclairements rouge et infra-rouge sont fournis aux plantes pendant de courtes périodes interrompant, en son milieu, la nuit longue de 15 h. Les plantes sont élevées en conditions dyspériodiques, c'est à dire non photo-inductives, de 24 h d'éclairement blanc; quand elles ont atteint le stade de 8 feuilles, une seule nuit longue suffit à provoquer la floraison[1]. Les plantes sont expérimentées à ce stade. Une interruption de la nuit longue par un éclairement rouge (30 sec, 15 min, ou 30 min) inhibe partiellement la floraison tandis que l'infra-rouge n'agit pas (Fig. 3). Ces résultats sont à rapprocher des observations faites en 1946 par Parker, Hendricks, Borthwick et Scully[4] en ce qui concerne l'effet des éclairements colorés interrompant la nuit longue. L'inhibition par le rouge n'est pas totale: une, deux, trois nuits interrompues ne permettent pas la floraison, mais quatre induisent une floraison tardive et de faible intensité (5 ou 6 fleurs par plante).

L'éclairement rouge est d'autant plus actif qu'il est fractionné en plusieurs périodes de très courtes durées (30 sec) au cours de la nuit longue. Ce dernier fait a été observé par Lincoln, Raven et Hamner[2] en 1956. Chez cette plante, contrairement à ce qui se

Bibliographie p. 406

produit chez de nombreuses autres espèces, la réversion de l'action du rouge par le proche infra-rouge n'a pas été observée.

Conclusions

Chez *Chenopodium polyspermum* l'éclairement bleu d'appoint au même titre qu'une longue période obscure est très efficace pour accélérer la floraison quand il est utilisé pour compléter l'éclairement de base blanc. L'éclairement rouge inhibe la floraison initiée par une seule nuit longue. Cette inhibition n'est pas totale et elle n'est pas supprimée par le proche infra-rouge.

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[1] R. JACQUES, *Colloq. intern. Photo-thermopériodisme*, Parma, 1957, p. 125.
[2] R. G. LINCOLN, K. A. RAVEN ET K. C. HAMNER, *Botan. Gaz.*, 117–3 (1956) 193.
[3] C. MEIJER, *Acta Botan. Neerl.*, 8 (1959) 189.
[4] M. W. PARKER, S. B. HENDRICKS, H. A. BORTHWICK ET N. J. SCULLY, *Botan. Gaz.*, 108 (1946) 1.
[5] J. A. J. STOLWIJK, *Mededel. Landbouwhogeschool Wageningen*, 54 (1954) 103.
[6] E. C. WASSINK ET J. A. J. STOLWIJK, *Koninkl. Ned. Akad. Wetenschap., Proc. Ser., C*, 55 (1952) 471.

Action des éclairements colorés sur la floraison de *Myosotis palustris*

Myosotis palustris est une plante de jour long absolue, c'est dire qu'elle ne fleurit jamais en journée courte de 9 h d'éclairement solaire. Nous étudierons ici l'action des éclairements colorés sur la floraison de cette plante, l'éclairement étant donné, soit comme appoint, soit en interruption de la nuit longue.

(1) *Eclairements d'appoint*

Si nous complétons un éclairement trophique obtenu avec 9 h de lumière blanche (2,000 erg/cm²/sec) par 15 h d'éclairement coloré de plus faible intensité, de l'ordre de 200 erg/cm²/sec, nous obtenons un effet de jour long avec le vert, le jaune, le rouge, l'infra-rouge et le blanc mais jamais avec le bleu.

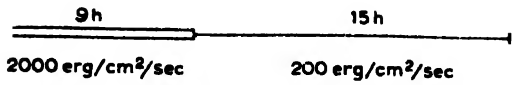
(2) *Interruptions de la nuit longue*

Si nous interrompons la nuit longue par 0.5 h d'un éclairement de faible intensité nous obtenons la floraison de *Myosotis palustris* avec les lumières rouge et blanche mais pas avec le bleu ni l'infra-rouge.

Quand ces éclairements blanc ou rouge sont suivis d'un éclairement de même intensité et de même durée, dans l'infra-rouge, leur effet est annulé. Il semble également que l'effet d'une interruption de la nuit longue par le blanc ou le rouge soit, sinon annulé, du moins fortement diminué par un éclairement bleu consécutif.

Conclusion

Chez cette plante l'effet "de jour long" provoqué par le blanc, le rouge clair et même le proche infra-rouge quand ils sont donnés en long appoint, aussi bien que



Noir: effet normal de jour court; Blanc, Vert, Jaune, Rouge, I.R.,: effet de jour long; Bleu pas d'effet de jour long, l'effet de jour court demeure.

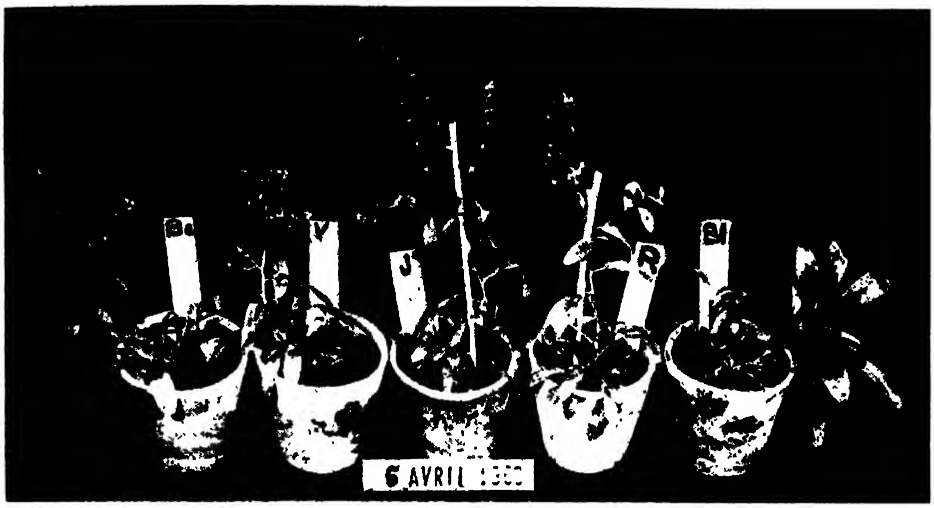


Fig. 1. Effet des éclairements d'appoint.

Blanc, Rouge; effet de jour long; Bleu, I.R.: pas d'effet de jour long, l'effet de jour court demeure.

Fig. 2. Interruptions de nuit induisant la floraison. N = noir, R = rouge, B = blanc.

l'effet de jour long dû à l'interruption de la nuit longue par un bref éclairement rouge, et son renversement en effet de jour court par un éclairement infra-rouge bref et immédiatement consécutif, sont des résultats maintenant classiques.

Mais il faut remarquer notamment les particularités de l'effet de la lumière bleue qui, contrairement à ce qui se passe chez d'autres espèces, agit de façon opposée à toutes les autres couleurs en lumière d'appoint prolongé et agit assez pareillement à l'infra-rouge en interruptions brèves, soit pour ne pas provoquer l'effet de jour long,

1/2h 1/2h

Blanc → I.R.: effet de jour long annulé, effet de J.C. Rouge → Bleu: effet de jour long atténué.

Fig. 3. Interruptions de nuit produisant un effet de jour court. N = noir, IR = infra-rouge, R = rouge, B = blanc.

soit pour annuler (mais en partie seulement) l'effet de jour long d'un éclair bref reçu préalablement en rouge clair ou en blanc.

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Influence de la lumière sur la germination des graines de *Phacelia tanacetifolia* Benth. *(Hydrophyllacées)*

La germination des graines de *Phacelia tanacetifolia* est inhibée par la lumière blanche (400–800 mμ)[3,4,8].

L'étude de l'action qualitative de la lumière a mis en évidence les faits suivants:

(1) *Influence de la lumière rouge sombre (700–800 mμ)*

La lumière rouge sombre inhibe fortement la germination. Le maximum d'activité est obtenu à 730 mμ. A cette longueur d'onde, en éclairement continu, l'intensité d'illumination nécessaire pour réduire la germination de 50% (i 50) est de 20 ergs/sec/cm^2. L'inhibition de germination est proportionnelle au logarithme de l'intensité.

(2) *Influence de la lumière rouge clair (600–700 mμ)*

La lumière rouge clair inhibe la germination avec un maximum à 660 mμ. A cette longueur d'onde, i 50 = 200 ergs/sec/cm^2.

(3) *Influence de la lumière bleue-violette (400–530 mμ)*

L'activité inhibitrice se répartit comme suit:

(*a*) 400–460 mμ: inhibition de la germination avec un maximum à 435 mμ. A cette longueur d'onde pour i = 400 ergs/sec/cm²,

$$Rg \frac{\text{pourcentage de germination à la lumière}}{\text{pourcentage de germination à l'obscurité}} = 0.54$$

(*b*) 460–500 mμ: pas d'inhibition. Pour i = 400 ergs/sec/cm², Rg = 1.

(*c*) 500–530 mμ: inhibition de la germination avec maximum à 505 mμ. Pour i = 400 ergs/sec/cm², Rg = 0.66

D'autre part, bien que les lumières rouge sombre et rouge clair inhibent la germination, leur antagonisme demeure: une température basse, voisine de 5°, est une des nombreuses conditions qui déterminent la germination des graines de *Phacelia* irradiées par la lumière blanche. Dans ces conditions, à la lumière blanche, 50% des graines germent. Si l'on intercale entre la source lumineuse et les graines un filtre laissant passer la lumière rouge clair, on obtient 82% de germination. Par contre, le fait d'intercaler un filtre qui ne transmet que la lumière rouge sombre inhibe quasi totalement la germination qui ne se produit qu'à 2%. Cette expérience met en évidence les faits suivants:

(*a*) La lumière rouge sombre inhibe fortement la germination quand elle agit seule. Cette même lumière, à intensité égale, a une influence inhibitrice moindre lorsqu'elle est associée à la lumière rouge clair.

(*b*) Les températures froides, qui sont capables de provoquer la germination des graines irradiées par la lumière rouge clair, n'ont aucune influence sur l'inhibition de la germination dûe à la lumière rouge sombre.

On peut réaliser la même expérience en utilisant des solutions d'acide gibbérellique à 100 p.p.m. L'étude faite sur les traitements susceptibles de faire germer les graines de *Phacelia* à la lumière nous a montré qu'il existait de nombreuses analogies entre le comportement de cette espèce de graine et celui de graines dont la germination est stimulée par la lumière blanche (exemple *Betula pubescens*[2]).

TABLEAU I

	Action de la lumière	*Traitements susceptibles de faire germer les graines à l'obscurité*
Betula pubescens (Black et Wareing)	La lumière blanche stimule la germination	– fortes tensions d'oxygène scarifications mécaniques températures froides – solutions d'acide gibbérellique à 100 p.p.m.
	Action de la lumière	*Traitements susceptibles de faire germer les grains à la lumière*
Phacelia tanacetifolia (Rollin[6,7] et Martin)	La lumière blanche inhibe la germination	– fortes tensions d'oxygène – scarifications mécaniques températures froides – solutions d'acide gibbérellique à 100 p.p.m. – trempages dans l'eau oxygénée – prétraitement pendant 2 h à 60°.

Bibliographie p. 410

Tableau I résume les résultats obtenus par Black et Wareing[2] sur *Betula pubescens* et par nous-mêmes sur *Phacelia tanacetifolia*[6,7].

Chez ces deux graines la présence d'un inhibiteur hydrosoluble et thermolabile a été mise en évidence.

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1 M. BAXTER JONES ET L. F. BAILEY, *Plant Physiol.*, 31 [5] (1957) 347.
2 M. BLACK ET P. F. WAREING, *Nature*, 180 (1957) 395.
3 K. BÖHMER, *Jahrb. Wiss. Botan.*, 68 (1928) 544.
4 M. EVENARI, *Radiation Biol.*, 3 (1956) 519.
5 S. ISAKAWA, *Botan. Mag. (Tokyo)*, 70 (1957) 831, 264 et 271.
6 P. ROLLIN, *Rev. gén. botan.*, 65 (1958) 440.
7 P. ROLLIN, *Compt. rend.*, 247 (1958) 1484.
8 E. H. TOOLE, S. B. HENDRICKS, H. A. BORTHWICK ET W. K. TOOLE, *Ann. Rev. Plant Physiol.*, 7 (1956) 299.

Session 2

ANIMAL PHOTOBIOLOGY

Chairman: M. H. Pirenne, Oxford (Great Britain)

Secretary: Torben Thiede, Copenhagen (Denmark)

Light-shock responses in crayfish

Light plays a very important role in the physical adjustments of many organisms to their environment. The responses to light by the larger metazoans, largely equipped with various motor appendages, have been studied extensively during the past century. These responses vary from animal to animal and under different stimulus conditions. However, a commonly observed phenomenon among a variety of animals is a sudden motor response to sudden change in incident light. For examples, clams contract their siphons[1], barnacles withdraw their appendages[2], tubicolous polychaete worms withdraw into their tubes[3], and sunfish show a backward movement reaction[4]. Wolf and Zerrahn-Wolf used the term "shock response" to describe this reaction of sunfish, and this term has been adopted to apply to the reactions of crayfish, which normally respond to sudden illumination with sudden changes in body tone or movements of appendages[5].

Individual crayfish of the species *Orconectes virilis* were placed in distilled water in a glass dish, which in turn, was placed within a translucent cylinder and a reflecting cone. Large recording electrodes which did not interfere with the movements of the crayfish were placed peripherally in the dish. These remote electrodes readily detect the bioelectrical potentials that accompany the motor responses. The potentials were amplified and recorded with a cathode ray oscilloscope (CRO) and camera. Tungsten filament lamps of various wattages producing a range of luminance values (see Table I) were used for stimulation. The animals were dark adapted for about 20–30 min before each test. During the periods of adaptation the animals became quiescent and hence there was little electrical activity on the CRO. Responses to sudden illumination showed up as electrical action potentials with amplitudes in the order of 50–100 μV which stood out clearly against the normally low background activity. Responses were not always clear and definite. Only responses that showed an obvious, sudden transient potential were characterized as true responses. These are listed in Table I.

The data indicate that only a little over one half of the stimulus situations were successful in eliciting responses. The lower luminance values were generally less effective in eliciting responses. Also the successful responses were much more variable in response time. In contrast the average response times for the luminance values above 15 mL fell within a narrow range. It appears that reflexive startle responses with minimal response times are involved at these higher intensities. At the lower intensities all responses may not be startle responses; undoubtedly some of them are simply motor responses to light, *i.e.* they are not emergency responses. And indeed in some experiments responses occur only after several seconds have gone by. For that reason some sort of time limit must be set up within which a response may be termed a shock response. This is discussed below. There is an obvious inverse relation between the stimulus strength and the average response time at the lower intensities. This was also reported by Stehr[6] for six species of aquatic arthropods with which he worked. The data of the present study and the data of Stehr do not follow the Bunsen-Roscoe reciprocity law, indicating that the responses are not a direct consequence of the

References p. 415

TABLE I

DATA ON SHOCK RESPONSES TO LIGHT IN CRAYFISH

Luminance in mL	*Number of tests*	*Number of responses*	*Response times (sec)*		*Percent responses*	*Average respons time (sec)*
1.7	11	4	1.77	1.98	36	1.69
			2.48	0.52		
5.3	17	8	0.84	1.29	47	0.91
			1.87	0.43		
			1.14	0.42		
			0.65	0.62		
10.8	14	8	0.64	0.20	57	0.38
			0.20	0.56		
			0.42	0.18		
			0.32	0.49		
15.6	7	3	0.15		43	0.23
			0.31			
			0.23			
31.1	9	3	0.13		33	0.26
			0.25			
			0.40			
51.8	5	4	0.14	0.26	80	0.20
			0.22	0.18		
84.6	8	6	0.20	0.13	75	0.26
			0.46	0.35		
			0.22	0.23		
121.0	4	3	0.20		75	0.22
			0.25			
			0.20			

primary photochemical action. Certainly the complexity of the central nervous system would introduce many potentially variable factors that become involved in the mediation from the initial photochemical event to the final motor responses.

Some recent experiments indicate that picrotoxin has definite effects on shock responses to light in crayfish[7]. Under a slightly different experimental design, 500 foot-candles average illumination produced 15 responses in 40 tests (38%) in control animals. The average reaction time was about 360 msec. Animals injected with picrotoxin responded 34 times in 35 tests (97%) and the average reaction time was 180 msec. In this particular study the time limit for a definite shock response was considered to be one second. Although this is a somewhat arbitrary limit, the studies reported above and the studies with picrotoxin indicate that one second is at present a reasonable limit to adopt for what is called a shock response.

A number of things tend to indicate that shock responses to light in crayfish are not rigid reflexes involving a minimum of neurological mediation. Firstly, there are large variations in response times, especially at the lower luminance values, even though these values are several log units above the intensity threshold for vision in crayfish[8]. Secondly, even at the higher stimulus intensities a response is not certain.

Finally, the experiments with picrotoxin indicate that a drug with central excitatory action can change both the percentage (probability) of response and the average response time, indicating that some modification of the central integration involved in shock responses is possible. In conclusion it is proposed that the general term "light-shock response" be used to describe the sudden motor responses rapidly following sudden changes in incident light. In crayfish these light-shock responses appear to be general enough to be treated as a definite phenomenon, but labile enough so as to indicate that they are not simple reflexes.

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1 S. HECHT, *J. Gen. Physiol.*, 1 (1919) 657.
2 W. VON BUDDENBROCK, *Z. vergleich. Physiol.*, 13 (1930) 164.
3 J. A. C. NICOL, *J. Marine Biol. Assoc., United Kingdom*, 29 (1950) 303.
4 E. WOLF AND G. ZERRAHN-WOLF, *J. Gen. Physiol*, 19 (1936) 495.
5 G. CAMOUGIS, *J. Cellular Comp. Physiol.*, 55 (1960) 189.
6 W. C. STEHR, *J. Exptl. Zool.*, 59 (1931) 297.
7 G CAMOUGIS AND S. L. WERNER, *Anat. Record*, 137 (1960) 345.
8 W. J. CROZIER AND E. WOLF, *J. Gen. Physiol.*, 23 (1939) 1.

The correlation of photobehavioural and retinomotor responses in the Pacific salmon

Certain behavioural responses of animals are results of the influence of the physico-chemical environment on the physico-chemical organism. The phototactic, migratory, feeding and schooling responses of the juvenile Pacific salmon (*Oncorhynchus*) may be correlated with their retinal responses.

Retinomotor responses

The retinal epithelial pigment, cones and rods in the salmon are capable of undergoing extensive photomechanical changes.

In the light, the pigment is fully dispersed, the cones are contracted and the rods are expanded. In the dark, the opposite happens. The movement of the cones and rods is due to the expansion and contraction of their myoids. The rate of change from dark-adapted to light-adapted condition and *vice versa* is also known[1-3]. Full light-adaptation occurs in about 20–30 min while it takes about 40–50 min for dark-adaptation to be completed.

Retinomotor responses elicited by low light intensities are similar to the process of dark-adaptation. When the light intensity decreases below their thresholds, the retinal pigment and visual cells commence migrating towards their dark-adapted states. When the light intensity falls below its threshold the pigment begins to contract and takes various positions ranging from the fully dispersed (light-adapted) state to the maximally contracted (dark-adapted) state, thereby regulating the amount of light absorbed inside the optic cup. This exposes the rods (whose myoids meanwhile contract, bringing them closer to the limitans), which have been shielded by the dispersed

References p. 417

pigment in the light-adapted state. The cones, simultaneously, migrate away from the limitans and eventually come to lie close to the contracted pigment. Thus, declining light intensity, when it reaches a certain level or levels, triggers these three responses.

Phototactic response

The retinae of newly hatched alevins do not undergo any photomechanical changes[2], although the alevins themselves are photosensitive as seen by their tendency to hide under stones, as a result of photo-negative response[4]. They become less photonegative and increasingly photopositive with age. This coincides with the greater development of the retinal elements resulting in their increased ability to respond to light, culminating in the photopositive emerged fry that is capable of marked retinomotor responses and possesses full visual acuity as shown by the feeding experiments[2].

Migration

The downstream migration of juvenile Pacific salmon which takes place at dusk is a combination of the fish's response to light and its individual behaviour pattern[5]. The retinae of sockeye and coho smolts as well as pink and chum fry are in the process of dark-adapting at the time of the commencement of downstream migration[3].

As the light intensity decreases, the fry of the migrating species *(O. nerka; O. gorbuscha; O. keta)* rise to the surface and either swim with the current or are displaced[4,6–8]. The mechanism of downstream migration is similar in both the fry and smolts. The coho *(O. kisutch)* fry do not show the same marked increase in activity at dusk. This appears to be the reason for their failure to rise to the surface and get displaced, except at times of high water when some displacement occurs[4]. The fact that the cone threshold of coho fry (10^{-1} ft.-c.) is lower than that of the other species (10^{0}ft.-c.), while their rate of dark-adaptation is very similar might be the reason for this difference in behaviour[2]. The fish commence migration as the light-intensity decreases below their cone thresholds and their eyes begin to dark-adapt resulting in a state of night blindness until the process is completed[6]. The light intensity decreases more rapidly than the eye can dark-adapt and due to the resulting semi-adapted state the fish lose their ability to maintain position with relation to some reference point and swim with or are displaced downstream by the current. When the eyes get completely dark-adapted they are able to perceive large objects and maintain position with reference to them and migration ceases or slows down considerably. This suggests that the states of retinal adaptations are responsible for the marked peak in migration at dusk. The longer the time for dark-adaptation, the longer the peak lasts. The peak in migration may also have definite survival value[4]. This peak is related not to the time of the day but to the light intensity[7]. This may be correlated with the fact that the retina of *Oncorhynchus* exhibits no diurnal rhythm[2].

The influence of temperature on migration[4] does not appear to be due to its effect on the retina of *Oncorhynchus*[9].

Feeding

The Pacific salmon is essentially a surface feeding fish which depends mainly on its vision for the location and capture of prey[4]. Active feeding ceases in the dark except

for the occasional chance capture of prey. As long as the light intensity is above the cone threshold, the ability to capture prey is at its maximum and when the light intensity is between the cone and rod thresholds, this ability decreases and is directly proportional to the logarithm of the light intensity. The method of capture of prey also changes. The fish stay at the bottom third of the aquarium and locate the prey by its silhouette. This change in the mode of capture indicates the inability of rods to resolve as the cones do and the loss of visual acuity in the scotopic visual field. With decreasing light intensity the difference between the shadow and background diminishes and when the light intensity is below the rod threshold the fish is unable to locate the prey and feeding stops[2].

Schooling

Sight is the primary requisite in the formation and maintenance of a school[1,10]. However, full acuity of vision is not necessary for this purpose. As long as the light intensity is above the rod threshold, fish are able to school and disperse only when the light falls below the rod threshold when even the shapes of large objects are not recognisable[2].

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1 M. A Ali, *Ph.D. Thesis*. Univ. British Columbia, 1958.
2 M. A. Ali, *Can. J. Zool.*, 37 (1959) 965.
3 J. R. Brett and M. A. Ali, *J. Fisheries Research Board Can.*, 12 (1958) 815.
4 W. S Hoar, *J. Fisheries Research Board Can.*, 15 (1958) 301.
5 W. S. Hoar, *Biol. Rev.*, 28 (1953) 437.
6 M. A. Ali and W. S. Hoar, *Nature*, 184 (1959) 106
7 J. G. McDonald, *M.Sc. Thesis*, Univ. British Columbia, 1956.
8 F. Neave, *J. Fisheries Research Board Can.*, 12 (1955) 369.
9 M. A. Ali, *Can. J. Zool.*, 38 (1960) 169.
10 M. H. A., Keenleyside, *Behaviour*, 8 (1955) 183.

Wirkung optischer Strahlung auf das vegetative Regulationssystem*

Bereits 1948 wurde von uns zunächst der Nachweis erbracht, dass gleichzeitige Bestrahlung mit UV + IR den Stoffwechsel bei Säugetieren und Vögeln beeinflussen kann[1-6]. Es wurde gefunden, dass solche Wirkungen immer dann auftraten, wenn UV-Strahlung unterhalb 330 nm zusammen mit IR-Strahlung über 3000 nm aufgestrahlt wurde. Ausserdem ist dabei entscheidend, dass der Quotient UV : IR in den Grenzen zwischen 1 : 25 bis 1 : 300 liegt. Schon damals konnte es wahrscheinlich gemacht werden, dass diese Wirkungen über das vegetative Nervensystem verlaufen. Die Abb. 1 zeigt den Stoffwechsel einer weissen Maus nach Bestrahlung mit einer Osram-Ultravitalux-Lampe. Abb. 2 zeigt die Stoffwechselsenkung bei Hühnern nach Bestrahlung mit UV + IR. Es wurde also damit erstmals der Nachweis erbracht, dass es Strahlenwirkungen gibt, die spezifisch das vegetative Nervensystem beeinflussen.

* Herrn Professor Dr. Hermann Giersberg zum 70. Geburtstag gewidmet.

Literatur S. 421

Durch die zentrale Stellung dieses vegetativen Nervensystems war es naheliegend, dass mit Einflüssen auf viele physiologische Funktionen im Organismus bei einer solchen kombinierten Strahlung zu rechnen ist.

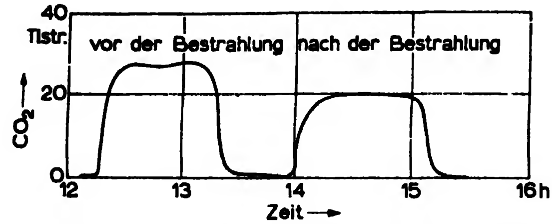

Abb. 1. Original-CO_2-Kurve einer Maus. Bestrahlung mit einer Ultravitalux-Lampe.

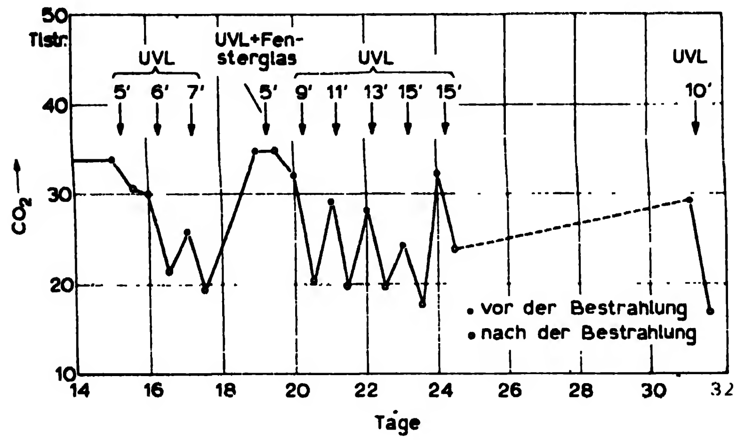

Abb. 2. CO_2-Kurve eines Huhnes. Bestrahlt mit einer Ultravitalux-Lampe (UVL).

Es muss betont werden, dass die Wirkung der UV + IR-Strahlung auf das vegetative Nervensystem auftritt, ohne dass der Erythem-Schwellenwert überschritten wird. Im Gegenteil, eine *optimale spezifische Wirkung* auf das VNS ist nur durch eine Bestrahlungsdosis zu erreichen, die unterhalb der Erythemschwelle liegt. Auf die günstigste therapeutische Strahlenbehandlung soll später noch etwas ausführlicher eingegangen werden.

Bereits bei den ersten Untersuchungen mit kombinierter UV + IR-Bestrahlung zeigte sich schon, dass neben der Wirkung auf den Stoffwechsel in hohem Masse auch der Blutkreislauf mit beeinflusst wird. Systematische Untersuchungen in dieser Richtung haben dann auch ergeben, dass durch die gleiche Strahlenkombination von UV und IR die Regulationsvorgänge des Kreislaufs weitgehend beeinflussbar sind. Durch kreislaufanalytische Messungen nach Wezler-Böger[7] bezw. Brömser-Ranke[8] haben sich diese Beobachtungen objektivieren lassen. Insbesondere vegetative Dysregulationen (Vegetative Dystonien) lassen sich durch systematische Behandlung mit dieser kombinierten Bestrahlung weitgehend günstig beeinflussen[9-12].

Bei dieser Strahlentherapie wird zweckmässigerweise so vorgegangen, dass zunächst bei den Patienten der Erythem-Schwellenwert bestimmt wird. Die ersten Bestrahlungen werden dann so dosiert, dass man etwa 10% unterhalb der Erythemdosis bleibt. Es können dabei sowohl Ganzkörperbestrahlungen wie Oberkörperbestrahlungen vorgenommen werden. Bei beiden Bestrahlungsverordnungen ist jedoch

stets darauf zu achten, dass der Kopf weitgehend ohne Strahleneinwirkung bleibt. Es hatte sich gezeigt, dass bei besonders strahlungsempfindlichen Patienten die Bestrahlung des Kopfes zu Schockwirkungen führen kann. Andererseits ist eine spezifische Wirkung der Strahlung auf das VNS stets gegeben, auch wenn nur der Oberkörper allein bestrahlt wird, sodass man also zweckmässigerweise, wie bereits betont, stets bei allen Patienten während der Bestrahlung den Kopf abdecken sollte. Die Bestrahlungen werden im allgemeinen jeden zweiten Tag vorgenommen, wobei mit fortschreitender Behandlung die Bestrahlungsdosis langsam erhöht werden kann. Da jedoch nicht wie bei den Bestrahlungen zur Erzeugung eines UV-Erythems eine

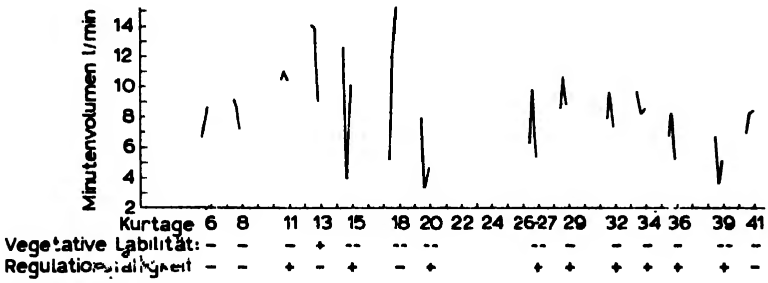

Abb. 3. Änderung des Minutenvolumens im Verlauf einer Kurbehandlung mit UV + IR.

allmähliche Erhöhung des Schwellenwertes auftritt, muss bei den kombinierten UV + IR-Bestrahlungen nicht unbedingt mit fortschreitender Behandlungsdauer die Dosis erhöht werden. Die Bestrahlungen können im allgemeinen wochenlang durchgeführt werden, ohne dass störende Begleiterscheinungen auftreten. Mindestens bilden solche Störungen die grosse Ausnahme.

Untersuchungen an einem grösseren Krankengut zusammen mit W. Amelung haben dies auf breitester Basis bestätigt. Die Abb. 3 zeigt die Änderung des Minutenvolumens im Verlauf einer Kurbehandlung mit UV + IR. Abb. 4 gibt die Änderung der anderen Kreislaufwerte: Elastischer Widerstand E', peripherer Widerstand W, der Pulsfrequenz sowie des Quotienten E'/W beim gleichen Patienten an. Das Zustandekommen der Kriterien für "vegetative Labilität" und "Regulationsfähigkeit" wird im nächsten Absatz erläutert werden. Mit Hilfe der objektiven Messung der Kreislaufwerte und deren Änderungen nach einer längeren Strahlenbehandlung lassen sich exakte Aussagen über den Verlauf einer Kurbehandlung oder eines Aufenthalts in einem Sanatorium bezw. einer Klinik machen. Dabei zeigte sich stets, dass sowohl vegetativ labile Patienten wie auch solche mit vegetativen Dysfunktionen in positivem Sinne auf die kombinierte UV + IR-Bestrahlung ansprachen.

Neben der therapeutischen Strahlenbehandlung insbesondere der vegetativen Dysregulationen konnte der Strahlenreiz mit UV + IR auch in einem objektiven Test zur Erfassung der momentanen vegetativen Regulationslage benutzt werden[13, 15]. Durch einen Strahlenreiz von 4 Min Dauer mit einer Osram-Ultravitalux-Lampe in 1 m Abstand auf den Oberkörper werden vegetative Regulationsvorgänge ausgelöst. Diese Funktionen spiegeln sich in den Änderungen der Kreislaufwerte wieder. Vergleicht man die Kreislaufwerte *vor* (a, Abb. 5.) und ***direkt nach*** dem Strahlenreiz (b, Abb. 5), so lässt sich daraus zunächst der Labilitätszustand des VNS beurteilen. Werden dann

Literatur S. 421

noch die Kreislaufwerte nach einer Erholungsphase *10 Min nach Ende* des Strahlungsreizes (c, Abb. 5) als Kriterium herangezogen, so lassen sich gleichzeitig Aussagen über die Regulationsfähigkeit bezw. Kompensationsfähigkeit machen. Die Abb. 5 zeigt schematisch die verschiedenen Möglichkeiten auf, die sich bei einem solchen Test ergeben können.

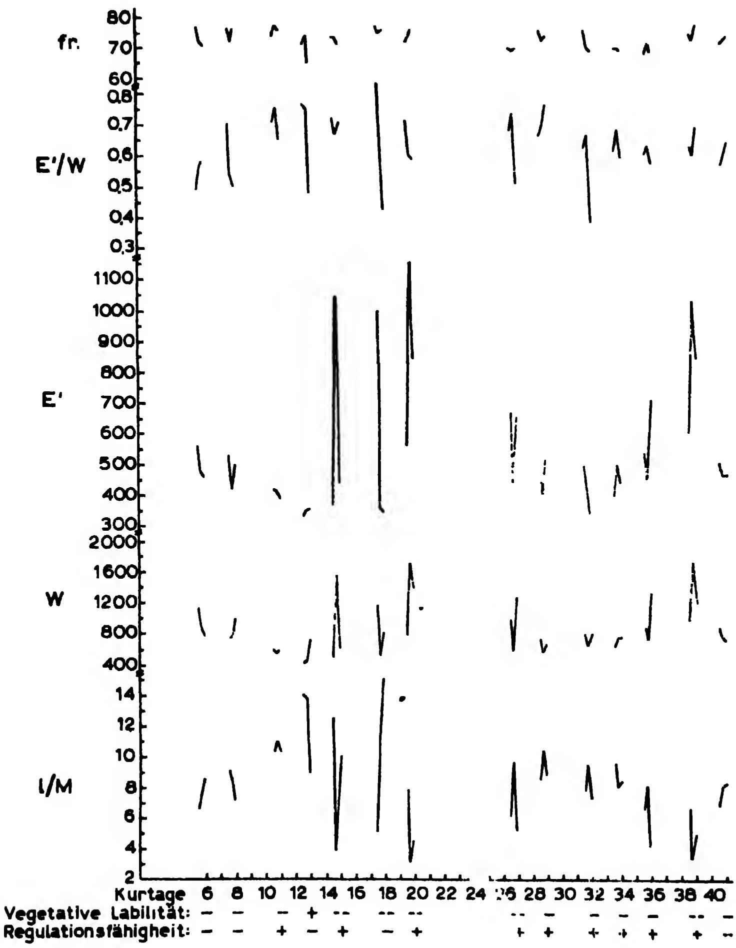

Abb. 4. Änderung des Minutenvolumens (L/M), des peripheren Widerstandes (W), des elastischen Widerstandes (E') und des Quotienten E'/W sowie der Frequenz (fr.) im Laufe einer Kurbehandlung.

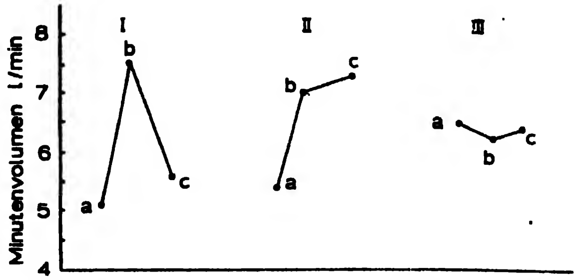

Abb. 5. Testbeispiele zur Beurteilung der "vegetativen Labilität" und der "Regulationsfähigkeit".

Zunächst kann folgender Fall eintreten, dass der Patient nach einem Strahlenreiz von 4 Min zunächst in seinen Kreislaufwerten sich stark verändert und z.B. das Minutenvolumen von etwa 5 l auf 7–8 l ansteigen kann, worauf es nach einer Erholungsphase von etwa 10 Min. wieder etwa den alten Wert einnimmt (I).

Zweitens besteht die Möglichkeit, dass zunächst ebenfalls eine starke Primärreaktion auf den Strahlenreiz einsetzt, dann aber nach der Erholungsphase nicht in gegenregulativem Sinne eine Kompensation stattfindet bis wieder in die Höhe des Ausgangswertes, sondern die Änderung des Kreislaufwertes in Richtung der Primärreaktion weiter fortschreitet (II). Die Kreislaufwerte (z.B. das Minutenvolumen) können sich bei der Primärreaktion erhöhen oder abfallen. Im II. Beispiel wurde der Fall einer Erhöhung angenommen.

Drittens kann die vegetative Lage so stabil sein, dass auf einen Strahlenreiz hin kaum eine Änderung der Kreislaufwerte, in unserem Falle des Minutenvolumens eintritt, wie dies der Fall III demonstriert. Im Fall III ist allerdings zu beachten, dass bei Extremwerten es sich auch hierbei manchmal um ein Erstarren des regulativen Systems handeln kann. Solche Fälle sind, wie unsere Erfahrungen gezeigt haben, therapeutisch oft auch sehr schwer anzugehen. Meist müssen solche Personen erst durch eine Art Schocktherapie aus dieser Erstarrung gelöst werden.

Insgesamt gesehen ergeben also, wie diese Beispiele zeigten, solche Belastungstests mit dem Strahlenreiz gute Einblicke in das regulative System. Entscheidend bei dieser Testung ist, dass für diesen Testreiz (kombinierte UV + IR-Strahlung) selbst nach wochenlangen Testungen niemals eine Gewöhnung eingetreten ist. Es ist dies eine Voraussetzung, die bei sonstigen Belastungsprüfungen selten erfüllt ist. Meist tritt nach einiger Zeit eine Gewöhnung ein, die das Ergebnis solcher Testungen dann etwas fraglich erscheinen lässt.

Die Untersuchungen zeigten, wie ich glaube hier zusammenfassend noch einmal betonen zu dürfen, dass durch die Kombinationswirkung von zwei Spektralbereichen, UV unter 330 nm und IR über 3000 nm, spezifische Wirkungen auf das vegetative Nervensystem auszuüben sind, die dem Kliniker vielleicht eine neue Methode in die Hand geben, mit geringstem Aufwand auf das nervös-humorale System einzuwirken.

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[1] R. G. A. LOTZ, *Verhandl. deut. Zool.*, 1948 (1949) 468.
[2] H. GIERSBERG UND R. G. A. LOTZ, *Z. Naturforsch.*, 3 b (1948) 349.
[3] R. G. A. LOTZ, *Z. Naturforsch.*, 3 b (1948) 357.
[4] R. G. A. LOTZ, *Z. vergleich. Physiol.*, 32 (1950) 228.
[5] R. G. A. LOTZ, *Arch. phys. Therapie*, 3 (1951) 357.
[6] R. G. A. LOTZ, *Strahlentherapie*, 86 (1951) 149.
[7] K. WEZLER UND A. BÖGER, *Ergeb. Physiol. biol. Chem. u. exptl. Pharmakol.*, 41 (1939) 292.
[8] PH. BRÖMSER UND O. RANKE, *Z. Kreislaufforsch.*, 25 (1939) 11.
[9] W. AMELUNG, R. LOTZ UND L. SCHMIDT, *Deut. Arch. klin. Med.*, 201 (1954) 568.
[10] W. AMELUNG, R. LOTZ UND L. SCHMIDT, *Arch. phys. Therapie*, 7 (1955) 221.
[11] W. AMELUNG, R. LOTZ UND G. ZETZSCHE, *Deut. Med. Wochschr.*, 81 (1956) 1635.
[12] R. MARTINI, *Umschau, Fortschr. Wiss. u. Tech.*, 57 (1957) 396.
[13] W. AMELUNG UND R. LOTZ, *Medizinische*, (1956) 1507.
[14] R. G. A. LOTZ UND W. AMELUNG, *Atti Congr. Intern. Idroclimatologia di Lacco Ameno, 1958*, Rizzoli, Milan, im Druck.
[15] W. AMELUNG UND R. G. A. LOTZ, *Atti Congr. Intern. Idroclimatologia di Lacco Ameno, 1958*, Rizzoli, Milan, im Druck.

Scanning movements of the stalked compound eyes in crustaceans of the order Stomatopoda

Mantis shrimps prowl over the ocean bottom along many of the world's coasts. These stomatopod crustaceans are active, firm bodied, powerful predators, ready for offense or defense with a folded, raptorial, second pair of thoracic legs, which suggest the prehensile first pair of legs on a praying mantis insect. Some stomatopods attain a body length of 30 cm, and can inflict a serious wound if handled or molested.

The stalked eyes of mantis shrimps have proved interesting for their ommatidia[1–9] and for their nervous connections[1,2,10–13]. The living animals, however, seem so visually aware of their unnatural surroundings in the laboratory that studies of their reactions to stimuli[14–16] must be interpreted with special care.

Working with *Squilla mantis* from the Mediterranean Sea at Naples, Demoll[15] showed the presence of both a binocular field of view and also a monocular stereoscopy. By sectioning a compound eye and examining the orientation of the individual ommatidia, Demoll accounted for the pattern of pseudopupils evident in the living eyes from various directions. *Squilla* responds differently to objects brought into its visual field from in front, from the side, and from above. The snatching response is most obviously related to objects above and in front of the stomatopod.

Schaller[16] found that illumination of *Squilla mantis* at any intensity higher than 100 lux inhibits feeding responses, and concluded that the locating and capturing of prey depends upon a combination of optical, tactile and chemical stimuli. Bolwig[14] tested *Gonodactylus glabrous* from the Indian Ocean near Lourenço Marques, Mozambique, and concluded that a negative response to light of daytime intensity, coupled with a positive response to body contact along lateral and ventral surfaces, serves to trap the animals under stones and other objects on the bottom through all hours of sunshine.

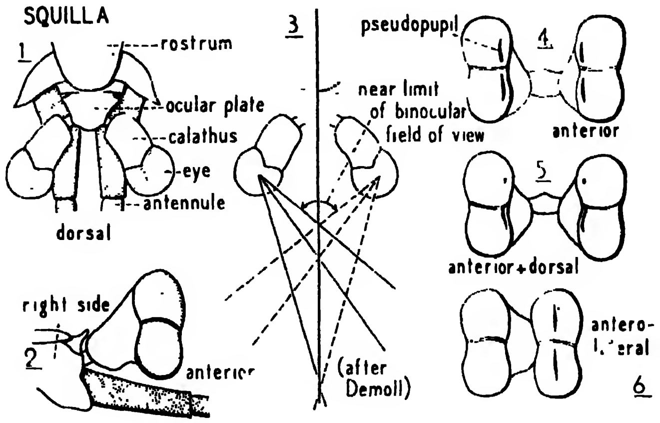
From examination of preserved material of several genera of stomatopod crustaceans, it became clear to us that the somewhat cylindrical compound eyes of *Squilla* (Figs. 1–2, 4–6) are very different from the more egg-shaped eyes of other genera, such as *Gonodactylus* (Figs. 7–9) and *Pseudosquilla* (Figs. 10–11). We therefore wished to compare living material belonging to these genera, and to observe in particular the pseudopupils while the animals were responding to stimulus objects near by.

MATERIALS

Through the kindness of Dr. Carl N. Shuster, Jr., we were able to study specimens of *Squilla empusa* collected at the Marine Laboratories of the University of Delaware. Living representatives of *Gonodactylus* and *Pseudosquilla* were encountered in shore waters of Bimini island in the Bahamas, near the Lerner Marine Laboratory of the American Museum of Natural History, where research facilities were generously made available to us. We are particularly grateful to the staff for making our short stay Bimini so productive.

The *Gonodactylus* were removed from the cavities of the loggerhead sponge in

which they hide during the day; each specimen was a clear, chlorophyll-green, almost without markings, but with brown eyes which did not change color with light and dark adaptation. The *Pseudosquilla* were captured running freely on the bottom in late afternoon; each specimen was greenish black, handsomely flecked with white chromatophores even on the dark brown eyes.



Figs. 1-6.

Fig. 1. *Squilla*, dorsal view of the ocular plate and associated structures, the animal facing the bottom of the page. Fig. 2. *Squilla*, same structures as in Fig. 1, seen from the right side. Fig. 3. The binocular field anterior to *Squilla* (after Demoll). Fig. 4 *Squilla*, appearance of pseudopupils from positions directly ahead of the animal and in the same horizontal plane (after Demoll). Fig. 5. *Squilla*, appearance of pseudopupils from directly ahead and about 20 degrees above the horizontal plane (after Demoll). Fig 6 *Squilla*, appearance of the pseudopupils from 55 degrees to the left of the animal's eyes in the horizontal plane, and hence beyond the field of view of the right compound eye (after Demoll).

RESULTS

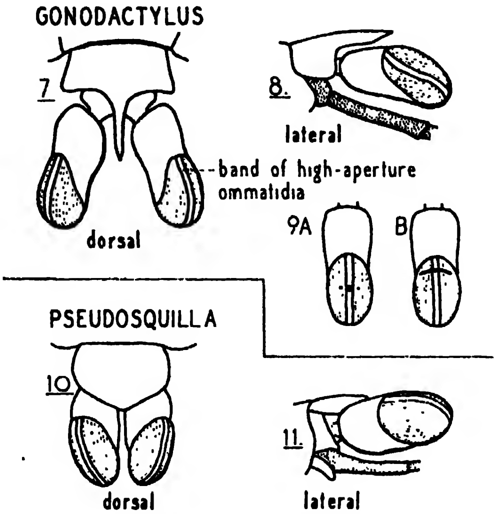
Demoll's findings with *Squilla mantis* from the Mediterranean[15] apply well to *Squilla empusa* from Delaware Bay (Figs. 1-6). But in both *Gonodactylus* and *Pseudosquilla*, we were astonished by the almost continual activity of the stalked eyes. These organs arise, as in the more generalized decapods, from an ocular plate. In stomatopods, however, the sclerotized portion ("*calathus*") of each eyestalk is attached by a very slender neck, differing greatly from the broad attachment seen in shrimps, crayfishes and crabs. The slenderness of the neck to the calathus permits the eyestalk to be swiveled more freely, and also allows it to rotate extensively — through perhaps 200 degrees — on its longitudinal axis. In addition, the ocular plate itself is remarkably flexible and undergoes nodding and swinging movements which add to the freedom with which the animal can change the position of its compound eyes.

Rotator muscles for the calathus are present in stomatopods. Muscles with this function have been described for the white shrimp *Penaeus setiferus*[17,18], but not in other decapods such as the crayfishes *Astacus* and *Cambarus*, the blue crab *Callinectes*, or the shrimp *Pandalus*.

In *Gonodactylus*, the rostral spine provides a landmark (Fig. 7) against which the movements of the eyes can be judged. Often the animal twists them to a position in which the axes of the eyestalks are parallel but at an angle of 45 degrees to the body axis, to right or left. In *Pseudosquilla*, this is less obvious, although comparable movements are made.

References p. 425/426

Both *Gonodactylus* and *Pseudosquilla* use the rotational possibilities of their eyestalks to aim at any object of special interest a definite band of ommatidia which separates the compound eye into a right and a left half of almost identical proportions (Figs. 7, 9). Due to the orientation of the ommatidia in the band and in adjacent areas, three distinct pseudopupils appear simultaneously in each compound eye (Fig. 9) when it is aimed at an observer.



Figs. 7–11

Fig. 7. *Gonodactylus*, dorsal view of ocular plate and associated structures, the animal facing the bottom of the page. Fig. 8. *Gonodactylus*, same structures as in Fig. 7, seen from the right side. Fig. 9. *Gonodactylus*, pseudopupils seen from the position toward which the animal aims its movable eye (A), and from slightly posterior to this position (B). Fig. 10. *Pseudosquilla*, dorsal view of eyestalks and rostrum, which conceals the ocular plate from above. Fig. 11. *Pseudosquilla*, same structures as in Fig. 10, seen from the right side.

By swinging and rotating the calathus for monocular or binocular viewing of a stimulus object, representatives of both genera will follow the position of a finger moving above the water surface or beside the transparent wall of a small aquarium in which they are creeping. When unstimulated, they employ the same movements to direct the band of ommatidia at objects on the bottom, and appear able to distinguish between non-food and food particles in this way. Only the latter lead to unfolding of the raptorial legs. Often the inspection of a sedentary worm or a stationary amphipod crustacean is carried on at extremely close range, as though the stomatopod were nearsighted and peering at details in order to comprehend the whole.

Each band is a narrow zone, six to seven ommatidia wide, in which the facets are slightly larger than those of ommatidia elsewhere in the eye. The ommatidia in the band are also shallower, and their axes more nearly parallel. Ommatidia beyond the band have smaller facets and usually more divergent axes, an exception to this being rather far back on each eye where the pseudopupil tends to become a transverse band (Fig. 9B).

All ommatidia we have seen in *Squilla*, *Pseudosquilla* and *Gonodactylus* are hexagonal, not square-facetted as stated by Gottsche[7].

DISCUSSION

Ommatidia in the longitudinal band of the compound eyes of *Pseudosquilla* and *Gonodactylus* seemingly correspond to the few in the slight angle between the two convex portions of the *Squilla* eye. The pseudopupils of *Squilla* are normally in a vertical line, whereas those of *Gonodactylus* and *Pseudosquilla* occupy this position only for objects at the side of the animal. The band ommatidia of *Gonodactylus* and *Pseudosquilla* are directed at objects of interest in a way that suggests the eye movements by means of which a vertebrate animal brings an image to the fovea for special study.

This analogy is misleading, however. The vertebrate fovea is a region of comparatively low sensitivity and high acuity. The stomatopod band ommatidia have relatively large facets, greater light-admitting power, lower numerical aperture, very slight divergence of axes, and correspondingly poorer resolution of details that could be passed to the central nervous system as contributions toward a visual mosaic. By oscillations of the calathus, the mantis shrimp may utilize the off-response in the band ommatidia to detect boundaries in a target. But for gaining any useful picture of form, it seems plausible that the ommatidia beyond the band — analogous to the receptor units in the vertebrate peripheral field — are the ones more suitable.

In *Squilla*, the two compound eyes are used simultaneously in relation to a binocular field below, in front of and above the animal. Demoll[15] regarded this as sufficiently definite to be worth plotting (Fig. 3). Both *Gonodactylus* and *Pseudosquilla*, by contrast, employ their compound eyes independently more often than together, and the monocular stereoscopic effect appears to be of greater significance.

The scanning movements of the compound eyes in *Gonodactylus* and *Pseudosquilla* appear to be a counterpart of the head-swinging activities of nun moth caterpillars[19] and the "hunting" actions of desert locusts[20]. They could be important also in keeping the central nervous system provided with a dynamic picture of the environment, and be analogous to the micronystagmus which is essential for our own appreciation of non-moving objects[21]. In a simpler way they may take the place of the four-channel information system with which a frog distinguishes an insect against a distracting, moving background[22].

Mantis shrimps give an impression of being far more alert than any lobster or crayfish, and at least as aware of their aquatic surroundings as the partially terrestrial ghost crab *Ocypoda* is of events in air. Some stomatopods demonstrate an additional possibility in movable stalked eyes, and may well prove to be the arthropods capable of a high degree of learning, perhaps comparable to that of cephalopod mollusks. On the basis of our experiences with *Gonodactylus* and *Pseudosquilla*, we would recommend them as particularly interesting subjects for new studies of animal behavior related to visual stimuli.

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[1] E. BERGER, *Arb. zool. Inst. Univ. Wien*, 1 (1878) 173.
[2] E. BERGER, *Arb. zool. Inst. Univ. Wien*, 1 (1878) 437.
[3] J. CHATIN, *Compt. Rend. et Mem. Soc. Biol.*, 28 (Mem. Ser. 6, vol. 3) (1876) 123.

4 J. CHATIN, *Ann. Sci. Natl. Zool.*, 6 (7) (1878) 1.
5 G. V. CIACCIO, *Mem. reale accad. sci. ist. Bologna, Classe sci. fis.*, 4 (5) (1894) 231.
6 G. V. CIACCIO, *Rendi. Accad. reale Sci. Istit. Bologna*, 1893-94 (1894) 105.
7 C. M. GOTTSCHE, *Arch. Anat. Physiol. u. wiss. Med.*, (1852) 483.
8 G. H. GRENACHER, *Untersuchungen über das Sehorgan der Arthropoden, insbesondere der Spinnen, Insecten und Crustaceen*, Vanderhöck und Ruprecht, Göttingen, 1879.
9 S. WATASÉ, *Stud. biol. lab. Johns Hopkins Univ.*, 4 (6) (1890) 287.
10 A. BETHE, *Pflügers Arch. ges. Physiol.*, 68 (10/12) (1897) 449.
11 B. HANSTRÖM, *Z. Morphol. u. Ökol. Tiere*, 23 (1/2) (1931) 80.
12 R. HESSE, *Z. wiss. Zool.*, 70 (3) (1901) 347.
13 E. RÁDL, *Z. wiss. Zool.*, 67 (4) (1900) 551.
14 N. BOLWIG, *Brit. J. Animal Behaviour*, 2 (4) (1954) 144.
15 R. DEMOLL, *Zool. Jahrb. Abt. Anat. u. Ontog. Tiere*, 27 (2) (1909) 171.
16 F. SCHALLER, *Z. Tierpsychol.*, 10 (1) (1953) 1.
17 J. H. YOUNG, *Tulane Univ. Stud. Zool.*, 3 (10) (1956) 169.
18 J. H. YOUNG, *U. S. Fish and Wildlife Serv. Fishery Bull.*, 59, No. 145 (1959) 1.
19 A. HUNDERTMARK, *Z. Forst- u. Jagdwesen*, 70 (5) (1938) 225.
20 G. K. WALLACE, *J. Exptl. Biol.*, 36 (3) (1959) 512.
21 R. W. DITCHBURN, D. H. FENDER AND S. MAYNE, *J. Physiol.*, 145 (1) (1959) 98.
22 J. Y. LETTVIN, H. R. MATURANA, W. S. MCCULLOCH, AND W. H. PITTS, *Proc. Inst. Radio Engrs*, 47 (11) (1959) 1940.

The relation of the frog's eye light-ERG and the X-ray-ERG

It is suggested that visible light and X-rays have an analogous effect on the retina. It was therefore possible to take an electro-retinogram from an isolated dark-adapted frog eye after irradiation with X-rays. This ERG has the same shape as a normal light ERG (Fig. 1).

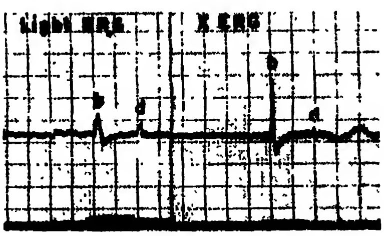

Fig. 1.

The X-ray dose used here was 27 R/sec, the voltage of the X-ray generator was 45 kV and the stimulation time was 5 sec. Doses as low as 2 R/sec also produce an X-ray-ERG. Sometimes we only recorded the "on" response, e.g. in a case where the stimulation cycle was 2 sec "on" and 5 sec "off". Attention may be directed to the decrease of the amplitudes of the waves during the successive stimulations — this decrease may probably be attributed to an injurious action of the X-rays. It is "the fatiguing effect" of X-rays, as it is called by Lipetz[1] and is a difference between the effect of X-rays and of visible light. If the periods between two stimulations are one minute or more, the "fatiguing effect" is not present. The amplitudes of the waves do not change in this case and it is possible that one minute is the time for complete recovery. With intervals under one minute the amplitudes always successively decrease until a certain minimal value has been reached. We never observed a

complete disappearance of the amplitudes, not even if we used a stimulation cycle of one second "on" and one second "off". In this case the amplitudes are all of the same height with the exception of the first which is much higher. A possible explanation of this fact may be that immediately after the first radiation dose an equilibrium is set up between "the fatiguing effect" and its recovery.

When this equilibrium has been reached a new dose of X-rays is incapable of causing a further decrease. If at this juncture a constant illumination with visible light from a minimal intensity is commenced, a further decrease of the amplitudes is observed. This means that visible light lowers the responsiveness of the eye to X-rays. The reverse is also true: X-rays diminish the responsiveness of the eye to visible light, in other words, irradiation with X-rays during the registration of a light-ERG decreases the amplitudes of this ERG.

These two facts, the lowering influence of light on the X-ray-ERG and the effect of X-rays on the light-ERG support the hypothesis that visible light and X-rays affect the visual system in the same way, *i.e.* by the energization of visual purple. The nature of "the fatiguing effect" is not yet clear. Lipetz[1] suggests denaturation of the proteins of the visual purple by the X-rays. It is known that X-rays produce ionization throughout the retina and this would be another possibility for the lowering effect; however, we should like to point out a third possible cause. De Robertis and Franchi[2] found subcellular vesicles in synapses of the retina and these are considered to be carriers of neurotransmittersubstances. We had concluded from other investigations that X-rays probably disrupt these vesicles. If this were also the case in X-ray irradiation of the eye, it would lead to reduced conduction of the impulses and this in turn to a diminishing of the responsiveness of the eye. However, further work is necessary before the precise mechanism can be elucidated.

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[1] L. LIPETZ, *Radiation Research*, 2 (1955) 306.
[2] E. DE ROBERTIS AND C. M. FRANCHI, *J. Biophys. Biochem. Cytol.*, 2 (1956) 307.

The structure and function of arthropod eyes

Nocturnal insects and crustacea have superposition eyes, which according to the classical descriptions of Exner, form, in the dark adapted condition, a single superposition image by the combined action of many adjacent ommatidia. By cutting the distal portion of the eye from the retina and substituting a clear liquid in the distal segment for the "Glaskörper" of the ommatidia (the "Glaskörper" consisting of retinal pigment cells and the crystalline stalk) Exner readily observed the superposition image in *Lampyris* and presumed similarly constructed eyes performed in like manner. Other eyes could not be tested by observation because lens components were not fused as in *Lampyris* and disintegrated on cutting into the eye.

By quick-freezing arthropod eyes in liquid nitrogen, the diopteric apparatus can be sectioned from the retinal elements and attached to a coverglass so that all the elements of the diopteric portion retain their proper position. After thawing, microscopic observation reveals image formation by the distal portion of the ommatidia.

Such sections were made of the light and dark adapted eyes of the crayfish *Orconectes virilis* and *Orconectes immunis* but none showed a superposition image. Light or dark adapted eyes did not appear to show any difference in image formation. Fixed and stained sagittal sections of *Orconectes* eyes showed an average divergence of adjacent ommatidial axes in mid-eye region of 2° 45′. The measured breadth of the visual field as observed through a single ommatidium was 32 mm at 20 cm distance which represents a visual angle of 9° 1′. As a measure of observed visual acuity, a figure of 1 mm width at 20 cm distance from the eye section (subtending an angle of 17′) was distinguishable.

The image of a single object (a figure 2 mm wide at 20 cm) when seen through the hemispherical segment of the eye of *Orconectes* appears repeated in a circular field of adjacent ommatidia. An average of 15 lineally adjacent ommatidia will form the same image however slightly laterally displaced in each one. As the maximal retinular width is only 2/3 the width of an ommatidium at retinal level, we may assume that only ten central lineally adjacent ommatidia can react to the same stimulus. This observation would indicate an effective visual angle for each ommatidium of approximately 20° 40′. Why this calculated visual angle should be double the measured visual angle is unresolved.

The eye of a lampyrid, *Photuris versicolor*, was similarly quickfrozen, sectioned, and prepared for microscopic observation. Again no noticeable difference appeared in image formation in light and dark adapted eyes. Unlike *Orconectes*, a clear superposition image was formed, but at a level behind (proximal to) the level at which the retina would have been. Average corneal-retinal distance measured in fixed material was 285 μ (with extremes at 171 μ and 410 μ). The average distance from cornea to superposition image was 355 μ (with extremes at 251 μ and 533 μ). The best preparation showed the superposition image at the maximum distance from the cornea. The average distance between retinal level and superposition image was 70 μ which represented about 25% of the average corneal-retinal distance.

Some *Photuris* eyes were prepared after the method of Exner by removal of the "Glaskörper" and pigments and the substitution of fluids with indices of refraction from 1.0 to 1.35 and as he observed a superposition image was formed. The average corneal-superposition image distance was in these cases 335 μ. If glycerine (*i.r.*-1.47) was substituted for the "Glaskörper" no superposition image was formed.

The angle of divergence of adjacent ommatidial axes in *Photuris* was computed from fixed material to be 1° 45'. The field of vision as observed and measured through a single ommatidium was 5° 1' (18 mm width at 20 cm distance). At this same distance an object 1 mm wide can be clearly discerned. In good sections 4 or 5 lineally adjacent ommatidia produce identical images indicating a computed field of vision for each ommatidium of 8° 5'. If only 2/3 of each visual field strikes the retinular area, the effective visual field would be 5° 45' which compares favorably with the direct measurement noted above.

These observations suggest that vision in the compound superposition eye does not depend upon the formation of a superposition image; it does not "see" the surrounding environment as a mosaic composed of coarse light and dark dots as has been frequently described. The structural arrangement of adjacent ommatidia with partial overlapping of their fields of view may be an evolutionary development permitting a more sensitive discernment of movement in the environment. The inability of the bee to differentiate between certain figures which are most obviously different to us would indicate that the receptor and interpretative portion of the arthropod eye has evolved with objectives other than those found in the vertebrate eye. Further studies on image formation with respect to the excitation of retinular cells in one and in adjacent ommatidia and a study of the patterns of distribution of reticular cell projection fibers in the optic ganglia would aid in clarifying the nature of vision in compound eyes.

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Hemmung der Photosensibilisation am Kaninchenohr

Viele Lichtdermatosen des Menschen beruhen auf einer photodynamischen Reaktion; sie sind dann ursächlich zu heilen, wenn der Sensibilisator erkannt und eliminiert wird. Leider gelingt diese causale Therapie nur bei wenigen Kranken. Es ist deshalb erforderlich, eine wirksame symptomatische Behandlung zu finden, mit der die photodynamische Reaktion an der Haut unterdrückt werden kann. Zu diesem Zwecke führten wir an Kaninchen folgende Photosensibilisationsversuche durch:

An den geschorenen Aussenseiten beider Ohrspitzen injizierten wir intracutan je 0.5 ccm Hämatoporphyrinlösung (Fertigpräparat Photodyn der Nordmarkwerke Hamburg). 45 Min. später wurden die Ohrspitzen 15 Min. lang mit dem UVA und sichtbaren Licht einer Kohlenbogen- bezw. Xenonhochdrucklampe aus 40 cm Abstand bestrahlt. Die Lichtsensibilisation zeigte sich schon 3–4 Stunden danach mit zunehmendem Erythem und starkem Ödem, welche nach 24 Stunden das *gesamte* Ohr einnahmen. Am Ort der stärksten photodynamischen Reaktion, am Quaddelareal,

entwickelte sich nach 3–4 Tagen eine Hautnekrose, die manchmal sogar zur Perforation des Ohres führte.

Da die photodynamische Reaktion nach Straub nur in Gegenwart von Sauerstoff stattfinden soll, wurde der Einfluss eines temporären Sauerstoffmangels auf die Photosensibilisation der Kaninchenhaut überprüft. Dazu unterbrachen wir die Blutzirkulation für 75 Min. an einem Ohr durch Kompression der Blutgefässe an der Ohrwurzel mit Hilfe einer gepolsterten Darmquetsche und zwar *vor und während*, — in einer weiteren Versuchsreihe *während und nach* der provozierenden Bestrahlung. Am anderen Ohr blieb die Blutzirkulation dagegen frei. Es zeigte sich danach am Ablauf der Lichtentzündung, an der Grösse des Nekrosebezirkes und der Perforation des Ohres ein deutlicher Unterschied zwischen abgeklemmtem und nicht abgeklemmtem Ohr. War die Blutzirkulation *während und nach* der Bestrahlung für 75 Min. unterbrochen worden, stellten sich nur geringe Schäden ein. Die Ischämie *vor und während* der Bestrahlung intensivierte dagegen die Lichtsensibilisation.

In weiteren Versuchsreihen wurde der Einfluss von reduzierenden Substanzen auf die photodynamische Reaktion im Rechts-links-Versuch am Kaninchenohr ausgetestet. Wir versuchten damit, die Photooxydation, bei welcher ja gewebsschädigende Peroxyde entstehen, zu hemmen. Nach der Injektion von 0.5 g Ascorbinsäure in die Zentralarterie des *einen* Ohres unmittelbar vor der provozierenden Bestrahlung lief die Lichtentzündung an *beiden* Ohren jedoch gleich stark ab. Ebenfalls war der Effekt von 0.2 g Cystein-Hydrochlorid intraarteriell unmittelbar vor der Bestrahlung nicht überzeugend. Dagegen wurde die Photosensibilisation durch 0.1 g Cystein — auf Gewebs-pH abgepuffert und unter das Quaddelareal injiziert -deutlich vermindert. Eine auffallend starke Hemmung der Lichtentzündung trat dann ein, wenn wir das Areal der Porphyrinquaddel und seine Umgebung an drei aufeinander folgenden Tagen täglich einmal mit 125 mg Dithioglycerin einrieben und erst danach bestrahlten. Das ölige Lösungsmittel des Bal übte dieses Effekt nicht aus. Die intraarterielle Injektion von 0.2 g Nikotinsäureamid, welches ja schon seit Jahren zur Behandlung von Lichterkrankungen des Menschen verwendet wird, schwächte die Lichtsensibilisation ebenfalls – wenn auch geringer als Bal — ab.

Den stärksten Hemmeffekt auf die Lichtsentzündung übte jedoch, nach unseren bisherigen Untersuchungen, das Calcium-Natriumsalz der Aethylendiamintetraessigsäure bei intraarterieller Injektion von 0.2 g aus. Hierdurch wurden nicht nur die nach der Lichtentzündung *bleibenden* Hautschäden, sondern auch schon die entzündlichen Symptome derselben, das Erythem und Ödem, überzeugend gemindert. Parallel laufende Versuche mit intraarteriellen Injektionen entsprechender Dosen von anderen Calciumsalzen wie Calciumgluconat, — chlorid und Calciumthiosulfat übten diesen Effekt nicht aus. Das Calcium-Natrium-EDTA hat sich auch bei der Therapie von Licht*sensibilisations*dermatosen, aber nicht bei Lichtallergien des Menschen allen anderen Calciumpräparaten und weiteren von uns erprobten inneren Medikamenten als überlegen gezeigt.

Wir haben hier nicht die Zeit, den möglichen Mechanismus zu diskutieren, wie die von uns im Kaninchenversuch ausgetesteten Substanzen die photodynamische Reaktion beeinflussen. Es soll das einer späteren ausführlichen Publikation vorbehalten bleiben.

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Natural photodynamic sensitivity in *Tubifex*

In 1955 Munro Fox and Taylor[1] described the hypersensitivity of the worm *Tubifex tubifex* to high oxygen concentrations. This aquatic worm lives naturally in the dark under hypoxic conditions. Increasing the oxygen supply kills the animal in a few days. The living in the dark combined with hypoxia suggested to us a hypersensitivity to light irradiation and this has been the subject of our study. For irradiation we used a super-high-pressure mercury lamp (Osram HBO 200) fitted in a reflector-house with a condenser lens in order to obtain a beam of high intensity. The worms were put into a perspex box with an arrangement to equilibrate the water with different gases. When the water containing the worms was equilibrated with pure oxygen, irradiation of the animals caused death in about three minutes. The beginning of the damage could be observed as an instantaneous increase of the motility of the worms gradually followed by an irreversible immobility. About 30 min after the irradiation lysis of the dead worms started, colouring the surrounding water yellow-brown. We carried out some control experiments to ensure that it was not the heat of the lamp which killed the worms.

When the worms were irradiated under anoxic conditions by equilibrating the water with nitrogen the irradiation had no visible damaging effect. The motility slightly increased but at the time that irradiation under hyperoxic conditions would have caused a 100% mortality all the worms were undoubtedly living. (Figs. 1 and 2).

Fig. 1. *Tubifex* at the start of irradiation. Left compartment: nitrogen equilibrium; right compartment: oxygen equilibrium.

References p. 432

This oxygen effect strongly suggests a photodynamic action and the presence of fluorescent substances. As early as 1913 Zielinska[2] described the presence of fluorescent substances in analogous animals *e.g.* *Eysonia*. In extracts of homogenated worms and in the living worm with U.V. microscopy we could detect a blue fluorescent substance but we did not analyse it.

Fig. 2. *Tubifex* 2 h after the end of irradiation. The worms in the nitrogen compartment (left) are quite normal, while those in the oxygen compartment (right) are dead and already show lysis, colouring the water with their pigments.

Using filters in the light beam we found that "killing" wavelengths were in the region of 630 mμ and of 360 mμ in the long-wave U.V. Possibly short-wave U.V. is still more effective.

The sensitivity of *Tubifex* for ionizing radiation is amazingly small in comparison to that for visible and U.V. light. X-ray irradiation with doses up to 40,000 R had absolutely no effect on the worms. It is possible to protect these animals against light irradiation by substances which are known to be chemical protectors against ionizing irradiation *e.g.* cysteamin, and protectors against oxygen poisoning *e.g.* cobalto and mangano salts[3].

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[1] H. MUNRO FOX AND A. E. R. TAYLOR, *Proc. Roy. Soc. Ser. B*, 143 (1959) 214.
[2] JANINA ZIELINSKA, *Bull. intern. acad. polon. sci., Classe sci. math. nat. Sér. B*, (1913) 511.
[3] F. DICKENS, *Biochem. J.*, 40 (1946) 145.

UV-Wirkung auf *Pandorina morum* in Abhängigkeit vom Entwicklungsalter

Für die Untersuchung der biologischen Wirkung ultravioletter Strahlen stehen die Mikroorganismen schon lange Zeit im Blickpunkt des Interesses, da es sich bei ihnen um relativ einfach organisierte Systeme handelt, die zur Beobachtung des Schädigungsablaufes nach einer Strahleneinwirkung besonders gut geeignet sind.

Zahlreiche Arbeiten sind erschienen über die primären und sekundären Schädigungen, die sie beeinflussenden Faktoren, ihre Abhängigkeit vom spektralen Bereich der Bestrahlung, über Mutationen usw. Einen umfassenden Überblick gab Giese[1].

Während all diese verschiedenen Gesichtspunkte der Strahlenwirkung intensiv untersucht wurden, blieb ein Faktor, der die Reaktionen der Zelle auf UV-Einwirkung entscheidend mitbestimmen kann, das Entwicklungsstadium bzw. das Zellalter, verhältnismässig unberücksichtigt. Bei einigen wenigen Objekten hat man Unterschiede in der Strahlenempfindlichkeit in Abhängigkeit vom Entwicklungsalter der Zelle gefunden. Gemeint sind hier immer die Sensibilitätsveränderungen während der

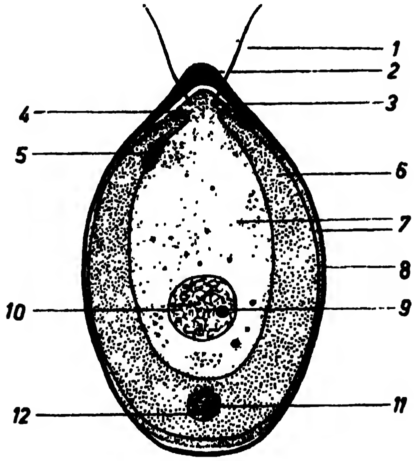

Abb. 1. Schema der Volvocaleszelle (verändert n. Pascher[6]).

1 Geissel	4 kontraktile Vakuole	7 Cytoplasma	10 Zellkern
2 Membranpapille	5 Stigma	8 Zellmembran	11 Pyrenoid
3 Basalkorn	6 Chloroplast	9 Nukleolus	12 Assimilationsstärke

Wachstumsphase der Zelle; die grössere Strahlenempfindlichkeit der Mitose ist ja allgemein bekannt. Für die bisher beobachteten Sensibilitätsänderungen während des Zellwachstums hat man nun einmal evtl. vorhandene Pigmente und zum anderen Veränderungen des physiologischen Zustandes der Zelle verantwortlich gemacht[2-5].

Für die hier vorliegende Untersuchung wurde ein Vertreter der Volvocales, *Pandorina morum*, verwendet, bei dem sich diese beiden Faktoren relativ gut voneinander trennen lassen. *Pandorina* sp. ist eine 16-zellige Kolonie, deren wichtigsten Organelle in der Einzelzelle die Abb. 1 zeigt. Die Alge wurde unter künstlicher Belichtung so

Literatur S. 435

gezüchtet, dass sich die Kolonien stets in der Nacht vom 3. zum 4. Tag teilten. Es standen also zu jeder Zeit Kulturen genau bestimmbaren Alters zur Verfügung.

Als Bestrahlungsquelle diente ein Quecksilberhochdruckbrenner vom Typ PRK 2 (= S450) des VEB Berliner Glühlampenwerk mit einem UG 11-Filter (2 mm) des VEB Jenaer Glaswerke Schott & Gen., das das sichtbare Licht bis auf einen kleinen Anteil im langwelligen Rot wegnimmt. Der Brenner wurde mit 220 V Gleichstrom betrieben. Die Strahlungsintensität betrug am Ort der Versuchsprobe stets $0.3 \cdot 10^6$ erg/cm² min.

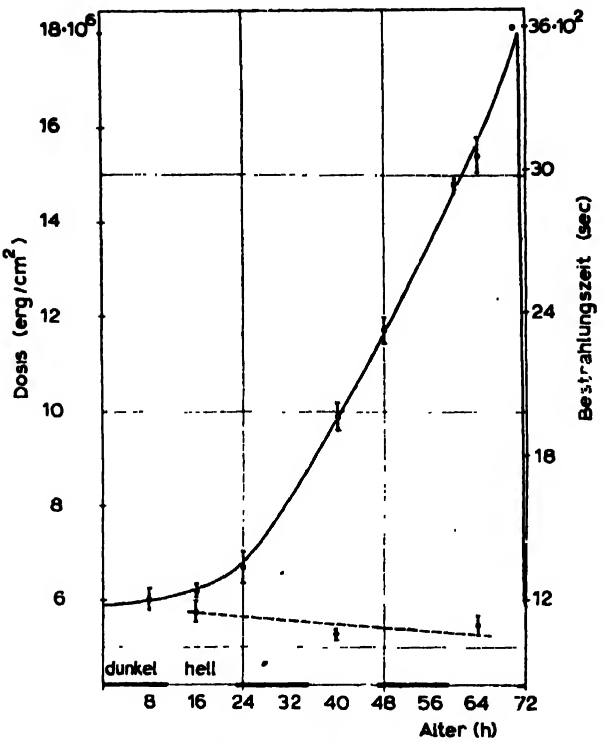

Abb. 2. Bestrahlungsergebnisse für *Pandorina morum* mit UG 11-Filter (2 mm). Die ausgezogene Kurve zeigt die mit dem Alter ansteigende Unempfindlichkeit und ihren Abfall während des Teilungsgeschehens. Die gestrichelte Kurve zeigt den Schädigungsgrad "Rotation am Boden".

Die Abb. 2 zeigt die mit dem Alter der Kolonie ansteigende Letaldosis und ihren schnellen Abfall innerhalb von *ca.* 2 Stunden auf den Ausgangswert während der Mitose. Die Schädigung der Lokomotion der Kolonie tritt bereits bei wesentlich kleineren Dosen ein und ist unabhängig vom Zellalter. Die Lokomotionsstörung äussert sich in einer Geschwindigkeitsabnahme der Bewegung bis die Kolonien nur noch am Boden rotieren und schliesslich ihre Geisselbewegung total einstellen. Eine Bewegungsbeschleunigung zu Beginn der Bestrahlung konnte nicht beobachtet werden. Bei zusätzlicher Verwendung der Filter WG 7 (2 mm), WG 6 (2 mm) oder WG 5 (2 mm) des VEB Jenaer Glaswerke Schott & Gen. steigt die Letaldosis so stark an, dass bei Verwendung des Filters WG 5 (2 mm) keine Abtötung innerhalb von 12 Stunden Bestrahlungszeit zu erreichen war (Abb. 3).

Das lässt sich dadurch erklären, dass in der Reihenfolge WG 7, WG 6 und WG 5 ein immer grösser werdender Anteil des kurzwelligen UV abgefiltert wird, so dass sich das wirksame Bestrahlungsspektrum immer mehr vom Absorptionsmaximum der

Thymonukleinsäure entfernt. Der wichtigste Erfolgsort der UV-Wirkung liegt also auch hier vermutlich im Zellkern.

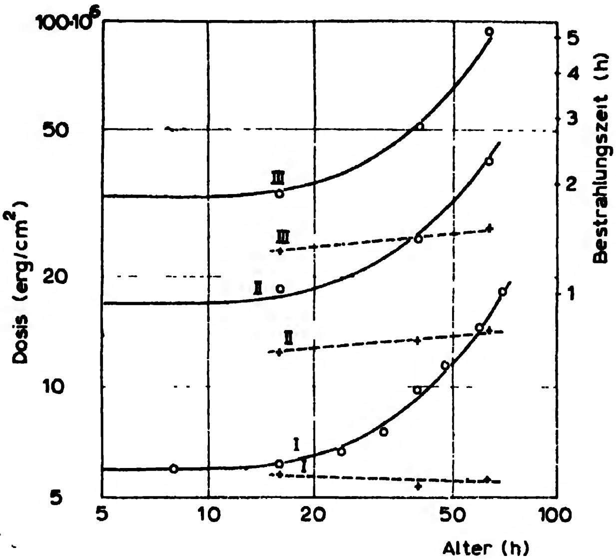

Abb. 3. Bestrahlungsergebnisse für *Pandorina morum* mit verschiedenen Filterkombinationen. Die ausgezogenen Kurven zeigen die Letaldosis und die gestrichelten den Schädigungsgrad "Rotation am Boden". I, UG 11-Filter (2mm); II, UG 11-Filter (2mm) WG 7-Filter (2mm); III, UG 11 Filter (2mm) + WG 6-Filter (2mm).

Bei allen Filterkombinationen blieb aber der altersabhängige Abfall der Sensibilität erhalten. Hierfür ist nun wahrscheinlich der Chloroplast verantwortlich, der wie ein optisches Filter den Zellkern umgibt. Mit dem Wachstum der Zelle nimmt seine Wandstärke und vermutlich auch die Chlorophyllkonzentration zu. Bei dem verwendeten Versuchsobjekt wurde nur das Ansteigen der Wandstärke des Plastiden gesichert. Durch die Zunahme der Schichtdicke gelangt immer weniger wirksames UV zum Kern, da ja das Chlorophyll an sich schon nur sehr gering UV durchlässig ist.

Zusammenfassend kann also gesagt werden, dass *Pandorina morum* im Laufe ihrer Entwicklung eine ständig ansteigende Resistenz gegenüber UV-Schädigung am Zellkern besitzt. Die Kolonie ist kurz vor der Teilung am strahlenunempfindlichsten und kurz danach am strahlenempfindlichsten. Dafür ist die Filterwirkung des Chloroplasten verantwortlich zu machen. Während das energetische Zentrum für die Geisselbewegung (Basalkorn), das durch kein optisches Filter geschützt wird, schon durch wesentlich kleinere Dosen altersunabhängig geschädigt wird.

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[1] A. C. GIESE, *Physiol. Zoöl.*, 26 (1953) 1.
[2] H. SCHREIBER, *Strahlentherapie*, 49 (1934) 541.
[3] P. S. TANG UND H. Z. GAW, *Chinese J. Physiol.*, 11 (1937) 305.
[4] T. HOUTERMANS, *Z. Naturforsch.*, 8b (1953) 767.
[5] G. C. MCLEOD UND J. MCLACHLAN, *Physiol. Plantarum*, 12 (1959) 306.
[6] A. PASCHER, *Die Süsswasserflora Mitteleuropas*, Jena, 1927.

Electrical responses in brain to photic stimulation*

Of interest is the manner in which organisms respond to the activation of their photoreceptors. For a number of years this has been studied in animals, and several authors[1-10] have investigated in particular the effect of photic stimulation on the cerebellum. Special problems occur with cerebellar recordings concerned with pharmacologic state of the animal as well as the methods and mechanics of recording responses.

In the present experiments, except for those testing certain anesthetics, surgery was performed under ether. The skull was opened over Larsell's[11] areas VI, VII, and VIII of the cerebellum and over the cerebrum in order to reach supratentorial optic structures such as areas 17 and 18 of the cortex, superior colliculus, lateral geniculate, and optic tract or chiasm. Tracheal and femoral cannuli were placed, the cat was oriented in a stereotaxic machine and maintained on small amounts of Flaxedil with artificial respiration. The right eye was taped closed. The left pupil was dilated with several drops of atropine sulfate and the retina stimulated during the experiment by a Grass PS2 photostimulator triggered by a Grass S4 stimulator. According to manufacturer's specifications $7.5 \cdot 10^5$ candle power was projected onto the retina.

The electrodes used were either pointed stainless steel wire insulated except for exposed tips of from 2 to 10 μ or 50 to 150 μ, or silver ball electrodes having exposed surfaces of about 500 μ diameter. Because of the use of differential amplification (common signal rejection) two electrodes were always used at once, one as recording "different" electrode, the other as "indifferent" electrode. There was also a separate "ground". Potentials picked up by the electrodes were passed through cathode-loaded input stages, Grass P 6 preamplifiers, visualized on Du Mont 322-A or 333 oscilloscopes and photographed by Grass C4F cameras. The different and indifferent electrodes were either bound together to form bipolar electrodes with distances between the tips of from 100 to 1000 μ in the case of microelectrodes or 400 to 1000 μ with silver ball electrodes, or the different and indifferents were used separately as monopolar electrodes at a distance from one another

Large electrodes tend to be less discriminating than small ones, the former tending to record activity at a distance rather than being restricted to events in the immediate area. Comparing electrodes of different sizes, it can be seen that bipolar microelectrodes will show no response, or an extremely small one, while silver ball bipolars placed in the same area of the same animal will consistently record a response. Also, the placement of the indifferent electrode is vital to the interpretation of results. Thus, in the unanesthetized animal bipolar microelectrodes will record no response from the cerebellum while monopolar microelectrodes with the different on the cerebellum and indifferent on bone over some optically active area will show a response. This response, however, is similar to that from the optically active area (area 17 in

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this case) and is not from the cerebellum. In this case the indifferent really acts as the different. Reversal of electrode leads will reverse polarity of response[12].

We have found that in cats anesthetized and maintained on Nembutal, Pentothal or ether no photic responses could be recorded from the cerebellum. Failure to obtain photically evoked responses in essentially unanesthetized animals maintained on Flaxedil was shown on a series of twenty-six cats in which one recording site was chosen and records made before and after chloralose. Other animals were systematically searched both on the surface of the cerebellum and in depth with essentially negative results before chloralose. The areas searched were those reported by other workers as being optically active in animals treated with α-chloralose. Directly following intravenous administration of chloralose evoked potentials appeared although several minutes were required for them to reach maximum amplitude and stabilize. Intravenous injection of Nembutal abolishes chloralose-induced cerebellar potentials, while only slightly diminishing responses of the optic cortex. It was also found that intravenous administration of strychnine will induce optic potentials in cerebellar cortex as will pentylenetrazol.

Simultaneous records from various optically active areas as well as from the cerebellum demonstrated the longer latency of cerebellar response. Since retinal delay varies inversely with light intensity[8] the intense light stimulus used here tended to make delay minimal. Inasmuch as experimental conditions vary from animal to animal we find it inaccurate to compare delay and transmission time or amplitude values except from multiple simultaneous records from the same animal. The method also provides a continuous check on the integrity of the optic system which is essential when recording from apparently inactive sites.

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[1] R. S. SNIDER AND A. STOWELL, *Anat. Record*, 82 (1942) 448.
[2] R. S. SNIDER, *A.M.A. Arch. Neurol. Psychiat.*, 64 (1950) 196.
[3] R. S. SNIDER AND A. STOWELL, *J. Neurophysiol.*, 7 (1944) 331.
[4] R. S. SNIDER AND E. ELDRED, *Anat. Record*, 100 (1948) 82.
[5] R. S. SNIDER AND E. ELDRED, *J. Neurophysiol.*, 15 (1952) 27.
[6] R. S. SNIDER AND E. ELDRED, *J. Comp. Neurol.*, 95 (1951) 1.
[7] W. P. KOELLA, *Federation Proc.*, 17 (1958) 89.
[8] W. P. KOELLA, *J. Neurophysiol.*, 22 (1959) 61.
[9] D. M. WHITLOCK, *J. Comp. Neurol.*, 97 (1952) 567.
[10] F. BREMER, *Rev. neurol.*, 87 (1952) 65.
[11] O. LARSELL, *J. Comp. Neurol.*, 99 (1953) 135.
[12] J. S. HAFT, C. A. HOVDE AND P. J. HARMAN, *Neurology*, (1960) in the press.
[13] C. M. BERRY, J. S. HAFT, P. J. HARMAN AND C. A. HOVDE, *Neurology*, 9 (1959) 845.

The testicular response of white-crowned sparrows to stimulatory photoperiods in ahemeral cycles*

Photoperiodic experiments employing ahemeral cycles, particularly with cycle lengths greater than 24 h, have been useful in studying the relative roles of light and darkness in photoperiodic responses and in examining the relationship between the photoperiodic responses and endogenous clocks[1]. As an example of the latter, Blaney and Hamner[2] have shown very nicely that the short-day photoperiodic control flowering in Biloxi soy bean requires that the photoperiod be synchronized with an endogenous 24-h rhythm. Danilyevskii and Glinyanaya[3,4] have used ahemeral cycles in investigations of the short-day induced diapause in the noctuid moth, *Acronycta rumicis*, and have shown clearly therewith that the response somehow has both dark and light requirements. Similarly, Wolfson[5] has investigated the long-day photoperiodic testicular response in *Junco hyemalis*. In this case, however, it is clearly evident that there is no dark requirement, thus confirming our earlier hypothesis for the similar response in *Zonotrichia leucophrys gambelii*[6]. The experiments reported in this paper represent primarily an examination of the question of a possible functional relationship of the photoperiodic testicular response to an endogenous 24-h rhythm in *Zonotrichia leucophrys gambelii.*

METHODS

The experimental birds were captured with Japanese mist nets from the population

Group	number of birds	number of cycles	Light-dark Cycle	K cycles^{-1}	K days^{-1}
VII-I	13	21-22	29	0.099 ±0.007	0.083 ±0.006
VII-II	13	25-26	22-29	0.088 ±0.005	0.084 ±0.005
VII-III	11	25	24	0.081 ±0.006	0.081 ±0.006
VIII-A	11	19-21	35	0.103 ±0.008	0.070 ±0.005
VIII-B	5	28	24	0.085 ±0.006	0.085 ±0.006

4 8 12 16 20 24 28 32
HOURS

light period · dark period · randomized dark period

Fig. 1. Testicular photoperiodic response in *Zonotrichia leucophrys gambelii* subjected to 18-h daily photoperiods in light-dark cycles of different durations. Responses are expressed by means of the testicular growth rate constant k; $k = (\log W_t - \log W_0)/t$ (ref. [7]) where W_0 is the initial (resting) testicular weight in milligrams, and W_t is the testicular weight in milligrams at time t in days or cycles (See text).

* The data on which this paper is based were in investigations supported by the Office of Naval Research, Contract Nonr-1520(00).

of *Zonotrichia leucophrys gambelii* which winters in the Snake River Canyon of southeastern Washington. From early winter they were held on daily photoperiods (*ca.* 8 h) sufficiently short to be non-stimulatory[7]. The basic experimental procedures and conditions including temperature, light sources, light intensity, and feeding were as described previously[7]. The photoperiods and dark periods for the experimental and control groups are shown in Figs 1 and 2. Groups VII-I, VII-II, and VII-III were

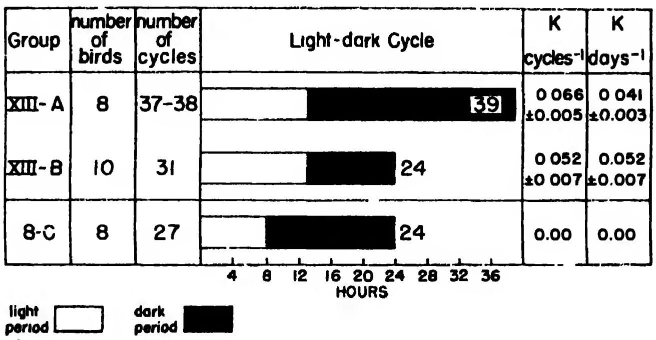

Fig. 2 Testicular photoperiodic response in *Zonotrichia leucophrys gambelii* subjected to 13-h daily photoperiods in 24-h and 39-h light-dark cycles. Group 8-C is a control group with the same light-dark ratio as Group XIII-A. For definition of *k*, see Fig. 1.

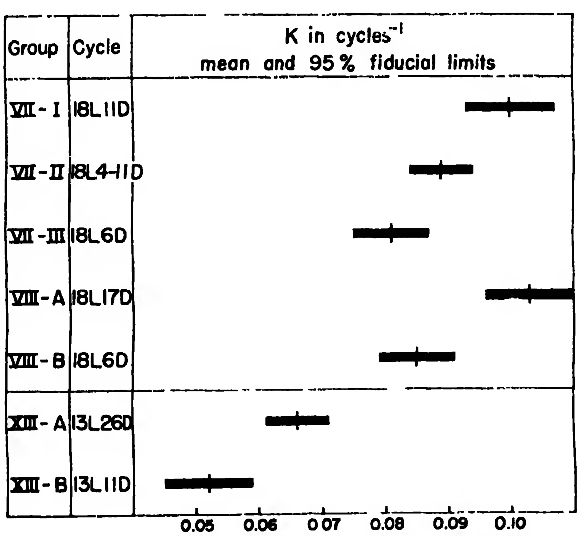

Fig. 3. Comparison of means and 95% fiducial limits for the experimental groups in Figs. 1 and The second column gives the number of hours of light (L) and dark (D) per cycle.

subjected to the photoperiodic treatment as indicated (Fig. 1) in December; groups VIII-A and VIII-B (Fig. 1) in January; and groups XIII-A, XIII-B, and 8-C in May. Controls taken at the beginning of each photoperiodic treatment had testes at typical winter resting state[7]. Group VII-II (Fig. 1) was subjected to a cycle with 18-hour photoperiods but with randomized total duration between 22 and 29 hours. Experi-

References p. 441

mental birds were killed after sufficient photoperiodic treatment to produce testicular weights of 100–200 mg.

The rates of response (Figs. 1 and 2) were expressed as the logarithmic growth-rate constant (k) of the testes[7]. Ordinarily with hemeral cycles k has the dimension of days^{-1}. With the use of ahemeral cycles, the question arises as to whether testicular growth, for comparative purposes, should be considered as a function of time (here, in days) or as a function of the number of cycles. Because there are cogent arguments for both, Figs. 1 and 2 give the constants with both dimensions. For purposes of comparison of responses the 95% fiducial limits of k (in cycles^{-1}) are recorded in Fig. 3.

I am indebted to Dr. Richard Parker for advice concerning statistical procedures, to Miss Betty Jane Johnson for the actual calculations, and to Mr. Donald F. Laws and Mr. Harold E. Cheyney for assistance with the experiments.

RESULTS AND DISCUSSION

A comparison of the mean rates of testicular development (Fig. 3) indicates clearly that this photoperiodic response in *Zonotrichia leucophrys gambelii* does not require a 24-h light-dark cycle or even an approximation thereof. This is also obviously the case with *Junco hyemalis* as indicated by the investigations of Wolfson[5]. The testicular photoperiodic response in these two species therefore appears to be very different in principle from the photoperiodically induced flowering in Biloxi soy bean in which an obviously close interaction with an endogenous 24-h rhythm is involved[2]. However, there may be two possible rationalizations with a hypothesis of interaction of a stimulatory photoperiod with an endogenous 24-h rhythm, assuming that such exists in these species. There is the possibility[8] that the endogenous clock is extremely susceptible to changes in frequency and phase imposed by the external *Zeitgeber*, the recurring photoperiod. There is the further possibility that the endogenous clock retains its 24-h periodicity but is reset by each photoperiod. (See ref.[9] for a discussion of resetting in this sense.) To me however, a simpler hypothesis is that the photoperiodic response mechanism has no important functional relationship to an endogenous 24-h cycle; instead it operates primarily as relatively simple functions of intensity, duration, and wave-length composition of the light periods[10]. This hypothesis, together with our observations that birds held on short days for many months beyond the normal time of testicular development fail to show testicular development, lead me to feel that there is no well-developed endogenous gonadal cycle in *Zonotrichia leucophrys gambelii*. The increasing day length in spring therefore probably should not be regarded as an important *Zeitgeber* in the sense of Aschoff[11]. This is in contrast with the role of photoperiodic control of gonadal cycles in domestic ducks for which the experiments of Novikov[12] and Benoit *et al.*[13,14] are perhaps best interpreted as indicating a crude endogenous cycle with the changing day length serving as a *Zeitgeber* in the sense of Aschoff[11]. However, despite the possible differences in its role with respect to annual gonadal cycles, the available evidence suggests that the basic photoperiodic mechanism itself in *Zonotrichia leucophrys gambelii* may be very similar to that of the domestic duck.

The data obtained in this investigation support strongly our earlier hypothesis[6] that the photoperiodic response is independent of the dark period, a conclusion which has been reached by Wolfson[5] for *Junco hyemalis*. A comparison of the responses of

groups XIII-A and 8-C emphasizes that this photoperiodic response is not a function of the light-dark ratio since this ratio is identical (2 : 1) in the two groups.

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1 E. BÜNNING, *Die physiologische Uhr*, Springer Verlag, Berlin, 1958.
2 L. T. BLANEY AND K. C. HAMNER, *Botan. Gaz.*, 119 (1957) 20.
3 A. S. DANILYEVSKII AND E. I. GLINYANAYA, *Doklady Akad. Nauk, S.S.S.R.*, 68 (1949) 785.
4 A. S. DANILYEVSKII AND E. I. GLINYANAYA, *Doklady Akad. Nauk, S.S.S.R.*, 71(5) (1950) 963.
5 A. WOLFSON, *Science*, 129 (1959) 1425.
6 D. S. FARNER, L. R. MEWALDT AND S. D. IRVING, *Biol. Bull.*, 105 (1953) 434.
7 D. S. FARNER AND A. C. WILSON, *Biol. Bull.*, 113 (1957) 254.
8 J. ASCHOFF, *Z. Tierpsychol.*, 15 (1958) 1
9 C. S. PITTENDRIGH AND V. G. BRUCE, *Photoperiodism*, AAAS Publ. No. 55 (1959) 475.
10 D. S. FARNER, *Proc. 19th Ann. Biol. Colloq.*, Oregon State College, (1958) 17.
11 J. ASCHOFF, *Studium Generale*, 8 (1955) 742.
12 B. F. NOVIKOV, *Akad. Nauk Ukrainskoi S.S.R., Trudy Inst. Zool.*, 10 (1953) 35.
13 J. BENOIT, I. ASSENMACHER AND E. BRARD, *Compt. Rend.*, 241 (1955) 251.
14 J. BENOIT, I. ASSENMACHER AND E. BRARD, *Compt. Rend.*, 242 (1956) 3113.

The intracellular distribution of phytochrome in corn seedlings*

A major advance in the field of photomorphogenesis is the recent isolation of the red-far-red reversible pigment *phytochrome*[1]. This pigment-protein was salted out from alkaline corn seedling extracts that had been centrifuged at 140 $k \cdot g$. Such sedimentation characteristics suggest that the complex is part of the soluble proteins of the cytoplasm.

We have found, however, that the phosphorylative capacity of *Avena* mitochondria is changed by red and by far-red irradiation of the plant[2]. These two radiation bands also reversibly alter the phosphorylative capacity of isolated animal mitochondria[2]. Phytochrome would thus appear to occur both in plants and animals as a mitochondrial component. Since knowledge of the location of phytochrome in the cell could be a clue to the locus and nature of its action, the intracellular distribution of the pigment complex was investigated.

METHODS

The fractionation procedure is outlined in Table I. It consisted of dispersing the plant material in a medium that would tend to maintain organelle integrity and yet incorporate the reducing agents used by Butler *et al.*[1]. Corn (*Zea mays* var. Marcross) was germinated and grown in the dark at 25° for 3 days. The seedlings were cut at the scutellum and dispersed in an Eppenbach colloid mill whose inner metal surfaces had been made water-repellent with silicone. Differential centrifugation was carried out in International refrigerated (with high speed attachment) and Spinco Preparative centrifuges. A Raytheon oscillator was used for the sonic treatment.

Organelles of plant cells intergrade in size and density[3], and the preparations listed in Table II are to be interpreted as *containing*, or *predominantly composed of*,

* This work was performed under the auspices of the U.S. Atomic Energy Commission.

References p. 443

TABLE I

FRACTIONATION SCHEME FOR PHYTOCHROME OF CORN SEEDLINGS

1. Blend plants several seconds with equal volume phosphate buffer, 0.2 *M* pH 6.5, containing 0.3 *M* sucrose, 0.01 *M* ascorbate, 0.01 *M* cysteine
2. Single pass through colloid mill, 50 μ passage
3. Filter through 100 mesh nylon and fractionate particulates by differential centrifugation
4. Sonic disintegration of each fraction at 10 kilocycles, 10′, 2°, and freeze in liquid N_2.
5. Disperse in phosphate medium and centrifuge 140 $k \times g$, 60′
6. Bring to 1/3 sat. with $(NH_4)_2SO_4$. Pack at 17 $k \times g$, 15′
7. Repeptize in 1/20 original volume 0.01 *M* phosphate buffer, pH 6.8
8. Centrifuge 17 $k \times g$, 30′
9. Irradiate equal volumes of supernatant in 655 and 735 mμ monochrometers, 5 min

the particulates indicated. Intact nuclei were detected by the acetic–orcein technique[4]. Mitochondrial preparations were positive to Janus green; they were also able to esterify adenosinediphosphate to adenosinetriphosphate[2], a phosphorylative capacity virtually absent in the microsomal preparation. Each disrupted cellular fraction was assayed for phytochrome by using difference spectrophotometry[1] We assume that phytochrome is a protein complex, of relatively low molecular weight, that is readily peptized at neutral pH.

Tungsten filament lamps and interference filters in monochrometers patterned after Withrow's[5] designs were the sources of red and far-red radiation. The far-red filter had a transmission of 50% at 735 mμ, with no radiation being detectable below 690 mμ in a Cary model 11M spectrophotometer. The red filter transmitted 75% at 655 mμ, 0.2% above 700 mμ, and cut off at 610 and 780 mμ. Incident energies were adjusted by varying the lamp voltage to yield 11 ergs/mm^2/sec at the red and 31 ergs/mm^2/sec at the far-red locus. Absorbancies of the irradiated solutions were determined in the Cary spectrophotometer using expanded scales. To maximize the differences between relatively low-order absorbancies, the phytochrome preparations were exposed to the two spectral bands simultaneously. They were then rapidly compared against each other in the spectrophotometer. This procedure also permitted automatic duplication in reversal experiments. A dim green *safe light*, with cut-offs at 500 and 550 mμ, was used when required in the period between irradiation and determination of absorbancy differences.

RESULTS AND DISCUSSION

Initially the method described by Butler *et al.* for the isolation of phytochrome from corn seedlings was repeated. The preparation obtained showed a $\triangle A_{655}-\triangle A_{735}$ of about $23 \cdot 10^{-3}$. This is well over the value of $5 \cdot 10^{-3}$ that, with our instrumentation, is the minimal value for significance, and corroborates the findings of the Beltsville group. Accordingly, their procedure was modified to that given in Table I in order to measure the concentration of phytochrome in recognized organelle groupings. Table II gives the absorbancy differences measured after exposure of the various intracellular fractions to red and far-red radiation. It is evident that phytochrome integrity is to a large extent retained during the fractionation procedure. This is demonstrated by the $\triangle A$ at the two wavelengths, as well as the reversibility of the system.

TABLE II

RED–FAR-RED ABSORBANCY DIFFERENCES [$\Delta(\Delta A)$] OF PHYTOCHROME FROM INTRACELLULAR FRACTIONS OF THE CORN SEEDLING

Centrifugation	*Description*	$(\Delta A_{655} - \Delta A_{735}) \times 10^3$ *FR vs. R*	*R vs. FR*	*FR vs. R*
10′, 100 × *g* ↓				
Supernatant	"Whole dispersion"	40	-41	35
10′, 1000 × *g*	Plastids, nuclei wall fragments	·5		
10′, 4,500 × *g*	Plastids, nuclei	8	-6	[illegible]
30′, 17,000 × *g*	Mitochondria	28	33	27
30′, 105,000 × *g* ↓	Microsomal	1	-4	3
Supernatant	Soluble protein	12	-15	

More significantly, the distribution of activity shows that the pigment complex was found both in the *soluble* protein fraction of the cytoplasm and in the mitochondrial fraction. The phytochrome concentration in the mitochondria was approximately double that found for the soluble fraction.

It is possible that phytochrome moves from the soluble cytoplasmic systems into organelle association when the pH is nonalkaline during fractionation. Alternatively, we would suggest that the alkaline conditions under which organelle integrities are not retained, and which are known to liberate proteins from bound complexes[6], could very well free phytochrome from an organelle association. Several responses to red and far-red are consistent with or support the interpretation of an *in vivo* association of phytochrome with mitochondria. These are adenosine triphosphate generation by mitochondria from irradiated *Avena* seedlings[2], phosphorylation by irradiated liver mitochondria[2], and the activation of phosphate esterification in lettuce seed[7]. The phosphorylation response of lettuce seed can be correlated with the photomorphogenic effects of the red spectral region on germination.

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1 W. L. BUTLER, K. H. NORRIS, H. W. SIEGELMAN AND S. B. HENDRICKS, *Proc. Natl. Acad. Sci., U.S.*, 45 (1959) 1703.
2 S. A. GORDON AND K. SURREY, *Radiation Research*, 12 (1960) 325.
3 D. R. GODDARD AND H. A. STAFFORD, *Ann. Rev. Plant Physiol.*, 5 (1954) 115.
4 J. H. TJIO AND T. T. PUCK, *J. Exptl. Med.*, 108 (1958) 259.
5 R. B. WITHROW, *Plant Physiol.*, 32 (1957) 355.
6 M. J. BLISH AND R. M. SANDSTEDT, *J. Biol. Chem.*, 85 (1929) 195.
7 S. A. GORDON AND K. SURREY, *Proc. Symp. Effects of Ionizing Radiation on Seeds*, Karlsruhe, Germany, Intern. Atomic Energy Agency, 1960.

The flash photolysis of visual pigments

The experiments to be described concern the reactions occurring in alkaline (pH 8.2–8.7) solutions of frog rhodopsin when they are exposed to intense flashes of light. The light source was a 400-joule xenon-filled discharge tube (Siemens SF E.22). The approximate flash duration was 4 msec. Flash intensity was controlled by interposing neutral gelatin filters between the flash tube and the rhodopsin solution. Solutions were prepared by extraction of acid-washed dark-adapted frogs' retinae with 2%

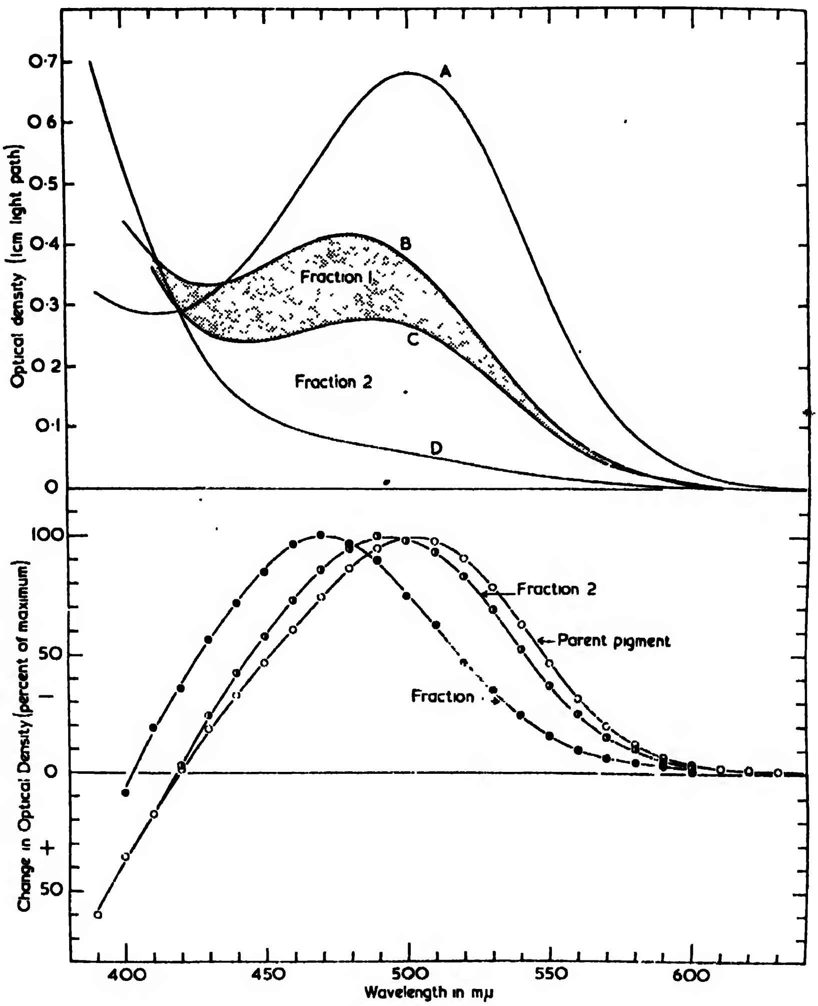

Fig. 1. Effect of flash-irradiating a solution of frog rhodopsin. Curve A — initial absorption spectrum: Curve B — absorption spectrum of initial photoproduct; this curve was measured from 600 to 400 mμ, the reading at 500 mμ was 30 sec after the flash: Curve C — after $1\frac{1}{2}$ h in darkness: Curve D — after exposure to normal white light (all absorption spectra measured at 25° on a Beckmann DK-2 automatically recording spectrophotometer — the temperature at the time of irradiation was 25°). The lower half of the curve shows the following difference spectra: parent pigment (O, D minus A); Fraction 1 (●, C minus B); Fraction 2 (◑, D minus C).

aqueous w/v digitonin. For experiments at temperatures below 0° glycerol (50 or 65% v/v) was added to these extracts, which were then adjusted to the required pH with an aqueous solution of sodium 5,5′-diethylbarbiturate. For experiments at normal temperatures the aqueous digitonin extract was adjusted to the required pH with a saturated aqueous solution of sodium borate.

Following exposure at 25° to a photoflash, a solution of frog rhodopsin contains two types of light-absorbing material. One type, Fraction 1, is thermally unstable and decays to indicator yellow (N-retinylidene opsin) during the course of about 1 hour. The other type, Fraction 2, is thermally stable and photosensitive. The presence of these two components in flash-irradiated rhodopsin solutions is illustrated in Fig. 1. Curve A is the initial absorption spectrum and curve B is measured within 30 sec of flash irradiation. During the next 1½ h the thermally unstable Fraction 1 decomposes, as shown by the fall in density to curve C measured 1 h later. Curve C is the absorption spectrum of a mixture of Fraction 2 and of indicator yellow derived from the thermal

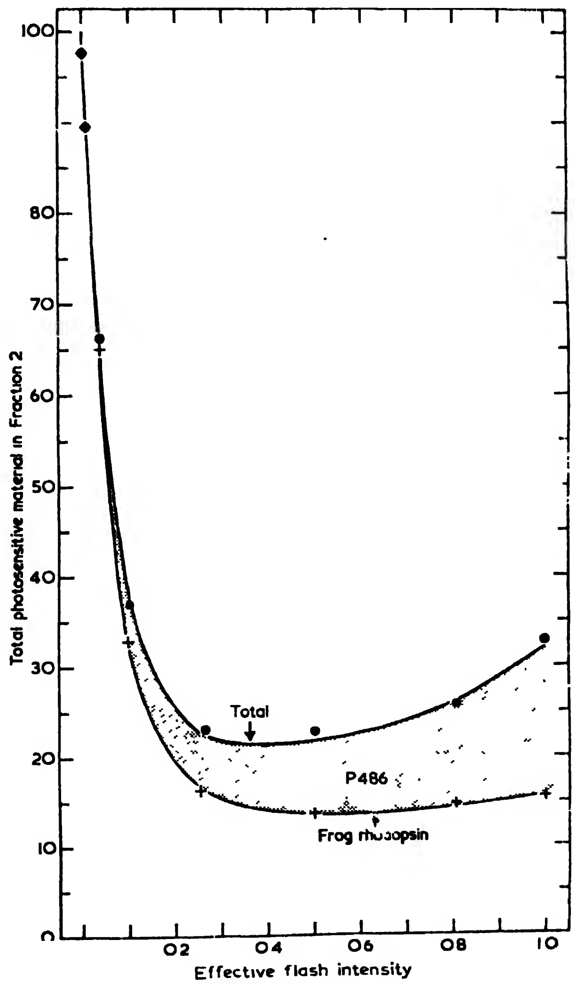

Fig. 2. Relationship between the amount of Fraction 2 produced and the flash intensity. The upper curve shows the total pigment in Fraction 2 (*i.e.* the summed amounts of P486 and frog rhodopsin), whilst the lower curve shows only the amount of the frog rhodopsin component (Temp. 25°).

References p. 447

degradation of Fraction 1. Curve D is measured after Fraction 2 has been completely bleached by exposure to a relatively weak white light (illumination about 25 ft.-c.).

Fraction 1 (cattle rhodopsin) has been studied by Wulff, Adams, Linschitz and Abrahamson[1], who investigated absorption spectrum changes in solutions of cattle rhodopsin during a period commencing a few μsec after flash irradiation. Fraction 2 has not been studied in detail, and its nature and mechanism of production is the principal subject of the present work.

As shown in Fig. 1 the λ_{max} of Fraction 2 is some 7–8 mμ below that of the parent rhodopsin *i.e.* it is at about 494 mμ compared with 502 mμ. Analysis of Fraction 2 by the method of partial bleaching[2] shows that it is a mixture containing approximately equal proportions of the parent rhodopsin and a new photosensitive pigment with λ_{max} at 486 $\pm$ 2 mμ. It is proposed to call this substance pigment 486 or P486; in this way it may be distinguished from a true *visual* pigment (*e.g.* frog rhodopsin or VP502).

"Fraction 2" designates *any* thermally stable photosensitive material remaining in solution after flash irradiation. If the flash intensity is reduced by means of a filter to 1/1000th of the unfiltered value, Fraction 2 is entirely rhodopsin and amounts to 97% of the pre-flash rhodopsin. In this case, Fraction 2 is merely unbleached rhodopsin. In Fig. 2 the curve marked "Total" shows the variation of Fraction 2 with flash intensity at 25°. The curve shows a minimum of 22% at about ½ full flash intensity. At the same time, the proportion of P486 rose from 0% at low intensities to

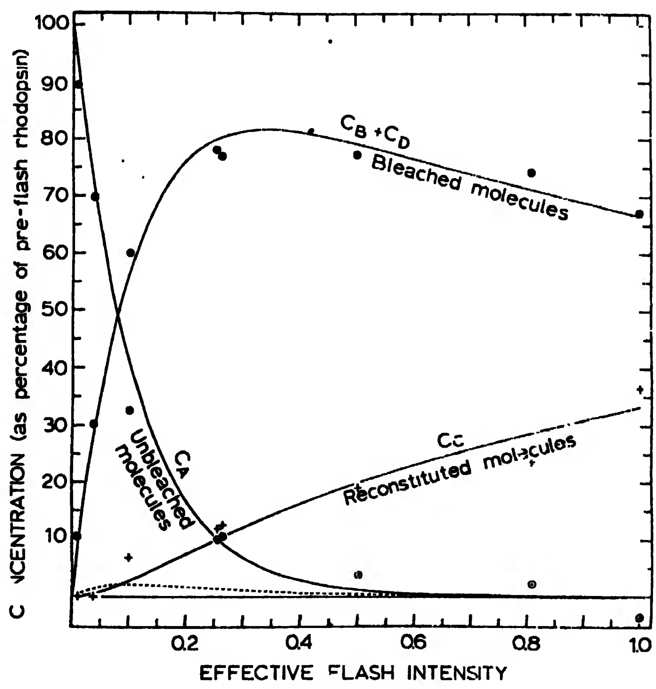

Fig. 3. Variation with flash intensity of the three types of substance present in a solution of frog rhodopsin 1 hour after flash irradiation at 25°. ⊙, unbleached molecules (the curve represents C_A); ●, bleached molecules (the curve represents $C_B + C_?$); +, regenerated molecules (a mixture of P486 and rhodopsin; the curve represents C_C). The dotted curve represents the concentration of hypothetical intermediate B at the end of the flash period.

between 46 and 57% at the highest intensity. As shown in Fig. 2, however, although the *proportion* of rhodopsin in the mixture decreased, the actual *amount* rose slightly towards the higher intensities. Thus although the "Fraction 2" of the lower intensities probably consisted entirely of unbleached parent rhodopsin, that of the higher intensities, in addition to containing flash-produced P486, contained also "flash-produced rhodopsin."

Hydroxylamine (0.01 *M*) does not affect the amount of Fraction 2 produced at any flash intensity. The quantity of Fraction 2 generated depends on the solution temperature, and, when produced by a full-intensity flash, rises by between 4 and 10% for each 10° drop in temperature from +40 to −40°. Viscosity apparently plays no part in this effect, since, for example, at +8° the same quantity of Fraction 2 is produced in solutions containing 65% v/v glycerol as in ordinary aqueous extracts.

The "Total" curve of Fig. 2 is the sum of two curves. One represents the amount of unbleached rhodopsin, and diminishes with rising flash intensity. The other represents the amount of a flash-generated mixture of P486 and rhodopsin, and increases with rising flash intensity. If it is assumed that this reconstituted material contains a constant proportion (47%) of P486 at *all* flash intensities, the amounts of unbleached rhodopsin, fully bleached rhodopsin and flash-generated photo-pigment may be obtained, and are shown as the points in Fig. 3. The curves of this Figure describe the experimental points and are calculated on the basis of the following scheme:

$$\underset{\text{(Rhodopsin)}}{\text{A}} \xrightarrow[k_1]{\text{light}} \underset{\text{(unstable intermediate)}}{\text{B}}$$

$$\text{B} \xrightarrow[k_2]{\text{light}} \text{C} \xrightarrow{\text{Slow}} \text{Reconstituted photo-pigments (P486 and rhodopsin)}$$

$$\text{B} \xrightarrow[k_3]{} \text{D} \xrightarrow[\text{via Fraction 1}]{\text{Slow}} \text{Bleached molecules (Indicator yellow)}$$

At the end of the flash period, *i.e.* when the solution contains only A, B, C and D, the concentrations of these substances may be calculated (Bridges[3,4]).

At present, the identity of the hypothetical state B is unknown. It may be the result of the primary photochemical event *i.e.* it is possibly an electronically excited state of the rhodopsin molecule. Again, it may be a new substance of the type found in the immediate post-flash period by Wulff, *et al.*[1].

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1 V. J. Wulff, R. G. Adams, H. Linschitz and E. W. Abrahamson, *Ann. N.Y. Acad. Sci.*, 74 (1958) 281.
2 H. J. A. Dartnall, *J. Physiol.*, 116 (1952) 257.
3 C. D. B. Bridges, *Biochem. J.*, 79 (1961) 128.
4 C. D. B. Bridges, *Biochem. J.*, 79 (1961) 135.

Photodynamic action of polycyclic hydrocarbons on isolated mitochondria in relation to their carcinogenicity

INTRODUCTION

A correlation between photodynamic action and carcinogenicity was first pointed out by Mottram and Doniach[1] testing 11 carcinogenic and non-carcinogenic polycyclic hydrocarbons and three sterol compounds on *Paramecia*. Maltoltsy and Fabian[2] found the same correlation in experiments on *Drosophila melanogaster* testing 3,4-benzpyrene, 20-methylcholanthrene and dibenzanthracene. Alexander and Fox[3] observed that the photodynamic degradation of polymethacrylic acid by 14 carcinogenic and non-carcinogenic polycyclic hydrocarbons and 4 stilbene derivatives was dependent on the degree of carcinogenicity. Garzia and Dansi[4] demonstrated that an alcoholic solution of triphenylformazane is more or less decolorized by indirect sunlight when different polycyclic hydrocarbons are added; the decolorization rate shows a correlation with the carcinogenic activity.

To avoid the objection that in the experiments mentioned above there can be no correlation between the very different actions compared, *e.g.* the production of tumors in vertebrates and death of a protozoon, we have studied the changes in human blood-serum when photo-oxidized in the presence of different polycyclic hydrocarbons. This investigation was carried out by paper electrophoresis; the number of hydrocarbons tested was 31. An impressive correlation was again observed between photodynamic action and carcinogenicity[5].

The photodynamic action of a carcinogenic hydrocarbon on isolated mitochondria was first studied by Graffi *et al.*[6,7]. These authors studied the behaviour of enzymes in isolated rat liver mitochondria in a glycerol solution of benzpyrene in salt solution which was irradiated by a quartz lamp, with filter passing 290–400 mμ, at 4°. The results showed that the succinic acid dehydrogenase activity was reduced to 1/100 of its initial value, cytochrome oxidase to 1/20, acid phosphatase to 1/20, alkaline phosphatase to 1/15, apyrase to 1/15; catalase remained unchanged. The replacement of molecular oxygen with oxygen-free nitrogen in the suspension prevented the enzymatic damage to a certain extent.

We investigated a similar photodynamic injury by measuring the variation of the optical density of mitochondrial suspensions. We observed that the photodynamic action of polycyclic hydrocarbons brings about a reduction in the turbidity of the suspensions. In experiments carried out with 13 carcinogenic and non-carcinogenic polycyclic hydrocarbons, a certain correlation was observed between photodynamic action and carcinogenicity.

The dramatic drop in turbidity brought about by 3,4-benzpyrene in the presence of light was completely prevented when the samples were exposed in vacuum, thus demonstrating the dependence of the phenomenon on oxygen. However, the addition of ATP, and reducing agents such as ascorbic acid, cysteine, glutathione, or thiourea in the presence of air failed to exercise any protecting action and even showed that these reducing agents bring about a marked drop in turbidity of mitochondria suspen-

sions in the dark and a greater drop in the light[5]. The swelling action by glutathione and cysteine in the dark is similar to that found by Lehninger and Schneider[8] under similar experimental conditions. We think that the failure of ascorbic acid, cysteine and other reducing agents to protect mitochondria suggests that isolated mitochondria are not suitable experimental material for studies of the mechanism of the photodynamic effect in cells. In addition, it should be noted that the results of studies on isolated mitochondria are influenced by the method of preparation, which, by itself, may have remarkable effects. However, the sure correlation found between photodynamic action and carcinogenicity suggested that it might be worth extending the investigation to a larger number of polycyclic hydrocarbons, as reported below.

METHODS

Livers from 24-h starved rats (250–350 g) of Wistar strain were homogenized in a cold room in a medium 0.50 *M* with respect to sucrose. After removing the readily sedimentable fractions by centrifugation at 600 *g* for 15 min, the mitochondria were isolated by centrifuging at 6,000 *g* for 30 min. The resultant pellet was resuspended in 0.46 *M* sucrose - 0.02 *M* tris(hydroxymethyl)aminomethane · HCl (tris) buffer, pH 7.4 or 8.6. 5 ml of the suspension was placed in cylindrical (1 cm diam) cuvettes of a Fisher electrophotometer containing 5 ml of a freshly prepared hydrocarbon pseudo-solution, using the same medium. The operation was carried out so as to obtain hydrocarbons at a final concentration of 10^{-5} *M* and mitochondria suspensions of 0.4-0.5 optical density at 520 mμ in a Fisher electrophotometer. The use of a medium consisting of 0.46 *M* sucrose was convenient in order to obtain resistent mitochondria.

A duplicate series of tests was prepared: one was kept in the dark and the other was exposed in a glass water bath to irradiation from a low-pressure quartz lamp: thus, wavelengths of less than 320 mμ were excluded. The intensity of irradiation, measured at the level of the cuvettes, was $33 \cdot 10^3$ erg/sec/cm². The suspensions were maintained at 20°.

The results were evaluated by means of the following calculation: if Δ^l_{r,x_0} and Δ^d_{r,x_0} are the differences of O.D. per cent of the controls (mitochondria alone) in the light and in the dark, and if Δ^l_{r,x_1,x_n} and Δ^d_{r,x_1,x_n} are those obtained in the presence of the experimental substances, it follows that

$$\Delta x_0 = \Delta^d{}_{r,x_0} - \Delta^l{}_{r,x_0}$$

and

$$\Delta x_1 \ldots x_n = \Delta^d{}_{r,x_1} \ldots {}_{x_n} - \Delta^l{}_{r,x_1} \ldots {}_{x_n}$$

Hence, the photodynamic activity, Δ_φ, of the substances, can be expressed:

$$\Delta_{\varphi 1} \ldots {}_{\varphi n} = \Delta x_1 \ldots x_n - \Delta x_0$$

6 compounds were tested in each experiment. In each case, both 3,4-benzpyrene and chrysene were included (the former being highly carcinogenic and highly active photodynamically, the latter non-carcinogenic and inactive). For every experiment, the results were plotted on a double co-ordinate graph, where the O.D. per cent of the control and the Δ_φ of the substances were plotted against the exposure time. A dotted

References p. 453

line was set on the time corresponding to the initial drop in turbidity of the control exposed to the light. The ordinate values of the intersections of this dotted line with the Δ_φ curves were used to calculate the relative Δ_φ, that is:

$$\Delta_{\varphi_r} = \frac{\Delta_{\varphi_{1 \ldots n}}}{\Delta_{\varphi_{bp}}} \times 100$$

where $\Delta_{\varphi_{bp}}$ is the Δ_φ of benzpyrene.

RESULTS AND DISCUSSION

The results of typical experiments are given in Figs. 1, 2, 3, and 4. Such graphs show that light alone damages mitochondria after 30–45 min illumination, although when active substances are present in the suspension, light produces an immediate effect. In all these figures it is apparent that 3,4-benzpyrene is the most effective, while chrysene does not produce any significant change.

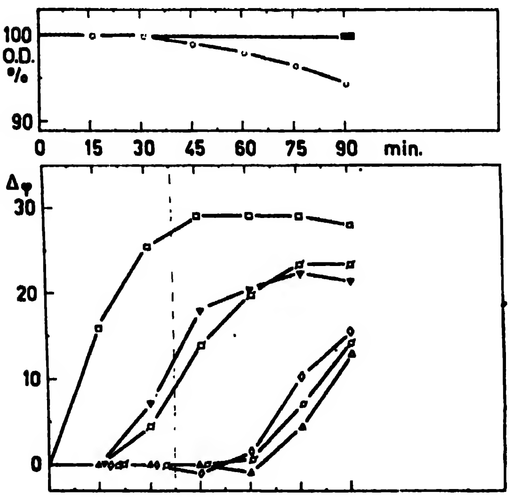

Fig. 1. Optical density of the controls and photodynamic activity (Δ_φ) of hydrocarbon plotted against the exposure time. The dotted line is set on the time of the initial drop in turbidity of mitochondria alone under light. ■ control and substances dark; ○ control light; □ 3,4-benzpyrene 10^{-5} M; ▽ pyrene; ⊓ anthracene; ◇ perylene; ⌀ phenanthracene; △ chrysene; initial O.D. = 0.43; pH = 7.4.

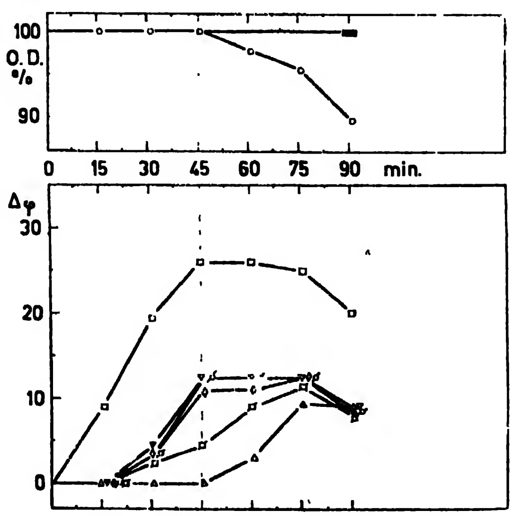

Fig. 2. Optical density of the controls and photodynamic activity of hydrocarbons as explained in Fig. 1. ■ control and substances dark, ○ control light; □ 3,4-benzpyrene 10^{-5} M; ▽ 1-methyl3,4-benzphenanthrene; ⌀ 7-Me-3,4 Bph; ◇ 6-Me-3,4-Bph; ⊓ 8-Me-3,4-Bph; △ chrysene; initial O.D. = 0.44; pH = 7.4.

The prompt response of 3,4-benzpyrene to the light compared with our previously observed lag-period (see [5]) is due to the lower initial optical density used in the present experiments. In fact, it is important in a comparative evaluation that the initial optical density should lie within the range 0.40–0.50. In the present series of experiments the significant time for a Δ_φ evaluation is that corresponding to the initial decrease in turbidity of the control exposed to the light. Such a time is within the range of 30–45 min; the change in optical density which takes place afterwards may

be interpreted as a phenomenon following the effect of light by itself on mitochondria[5].

It is of interest to point out that mitochondria suspended in 0.46 M sucrose–0.02 M tris, pH 7.4 are not affected at all by ageing at 20° in the dark even after 90 min, as far as the turbidity is concerned. At pH 8.6 (Fig. 4) only a negligible ageing effect is observed after 60 min and up to 90 min. For practical purposes therefore, no significant differences are observed in experiments carried out at these two different pHs (Figs. 2 and 4).

In Table I the values of Δq_r of the compounds tested are listed along with the approximate indications (–, +, . . . ++++) of their photodynamic action, including approximate indications of the data obtained in the photodynamic test on blood-serum (see Santamaria[5]), and the indications of carcinogenic action, as reported by Badger[9] and by Von Haam[10]. From this Table, containing the results of

TABLE I

Δq_r VALUES DETERMINED FOR VARIOUS COMPOUNDS TOGETHER WITH APPROXIMATE INDICATION OF PHOTODYNAMIC ACTIVITY ON MITOCHONDRIA AND ON BLOOD SERUM IN RELATION TO CARCINOGENICITY

	Photodynamic activity			
	On mitochondria			
Substances	Δq_r	Approximate indication	On blood serum	Carcinogenicity
3,4-Benzpyrene	100	++++	++++	++++
20-Methylcholanthrene	83	+++	+++	++++
1,2,5,6-Dibenzanthracene	6	* –	++	++
Anthracene	33	* +	–	
Phenanthrene	0	–	–	–
Chrysene	0		–	–
Pyrene	40	* ++	–	
Perylene	0		–	–
1,2,5,6-Dibenzacridine	23	+		±
1,2-Benzanthracene	38	+(+)	+	±
1'-Methyl-1,2-Benzanthracene	59	* ++	–	(b)
2'-Methyl-1,2-Benzanthracene	82	* +++	±	(b)
3'-Methyl-1,2-Benzanthracene	95	* ++++	–	(b)
4'-Methyl-1,2-Benzanthracene	20	* +	±	–(b)
3-Methyl-1,2-Benzanthracene	84	* +++	±	+ (a) + (b)
4-Methyl-1,2-Benzanthracene	84	+++	++	+ (a) ++ (b)
5-Methyl-1,2-Benzanthracene	100	++++	++	++(a,b)
6-Methyl-1,2-Benzanthracene	82	+++	+	+(a) ++(b)
7-Methyl-1,2-Benzanthracene	56	++	±	+(a) –(b)
8-Methyl-1,2-Benzanthracene	85	* +++	* ++	+(a) –(b)
9-Methyl-1,2-Benzanthracene	38	+(+)	+(+)	++ (a) + (b)
10-Methyl-1,2-Benzanthracene	56	++	* ±	+++(a) ++(b)
9,10-Dimethyl-1,2-Benzanthracene	23	* +	* +	++++(a) ++ (b)
1-Methyl-3,4-Benzphenanthrene	48	++	++	++(a,b)
6-Methyl-3,4-Benzphenanthrene	42	++	±	+(a) – (b)
7-Methyl-3,4-Benzphenanthrene	48	++	+	+ (a,b)
8-Methyl-3,4-Benzphenanthrene	17	±	+	+ (a) ++(b)

(a) Badger, 1948; (b) Von Haam, 1958

* Exception to the correlation between photodynamic action and carcinogenicity.

References p. 453

a study carried out with 27 polycyclic hydrocarbons, it is apparent that the correlatio between photodynamic action and carcinogenicity is greater in the test on blood serum (3 exceptions), and less evident in the case of the test on mitochondria (10 exeptions).

In the test on mitochondria the exceptions are non-carcinogenic compounds which display a photodynamic action; only in the case of 9,10-dimethyl-1,2-benzanthracene does a highly carcinogenic substance show a low photodynamic effect (also on blood-

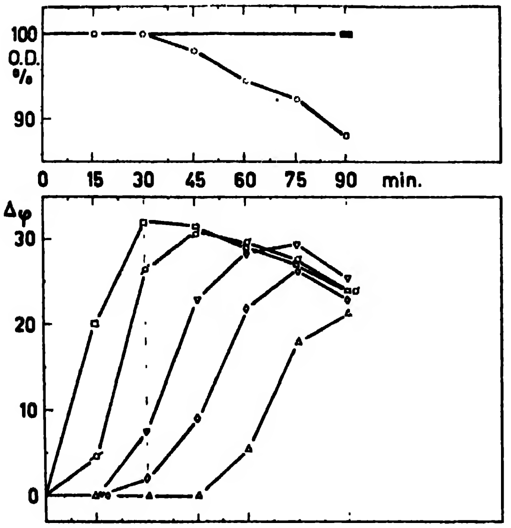

Fig. 3. Optical density of the controls and photodynamic activity of hydrocarbons as explained in Fig. 1. ■ control and substances dark; ○ control light; □ 3,4-benzpyrene 10^{-5} M; ø 20-methylcholanthrene; △ 1,2-5,6-dibenzacridine; ◇ 1,2-5,6-dibenzanthracene; ▽ chrysene; initial O.D. = 0.45; pH = 7.4.

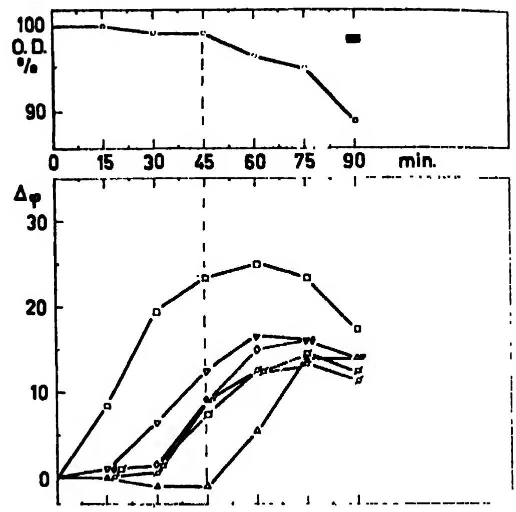

Fig. 4. The same experiment as in Fig. 2 pH 8.6. ■ control and substances dark; control light; □ 3,4-benzpyrene 10^{-5} M; 1-methyl-3,4-benzphenanthrene; ◇ 6-Me-3,4-Bph; ø 7-Me-3,4-Bph; 8-Me-3,4-Bph; chrysene; initial O.D. = 0.40.

serum). Upon the assumption that the photodynamic phenomenon is dependent on an association of the substance and the substrate, it would seem that quite a number of non-carcinogenic hydrocarbons combine with the mitochondrial structure. This general consideration, quite apart from the problem of photodynamic action, is somewhat limited by the observation that studies on isolated mitochondria are influenced by the mode of their preparation, which, as we have already indicated, may have remarkable effects.

On the whole, it seems to the writers that the blood-serum test is at present the one which lends most support to the hypothesis that the photodynamic property of polycyclic hydrocarbons is important as an aspect of carcinogenic action.

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* The authors are indebted to Prof. Melvin S. Newman (Department of Chemistry, Ohio State University) for his gift of the monomethyl substituents of 1,2-benzanthracene and of 3,4-benzphenanthrene.

J. C. MOTTRAM AND I. DONIACH, *Lancet*, 234 (1938) 1156.
G. MALTOLTSY AND G. FABIAN, *Nature*, 158 (1946) 877.
3 P. ALEXANDER AND M. FOX, *First Intern. Photobiol. Congress, Amsterdam, 1954*, Veenman, Wageningen, p. 336.
4 A. GARZIA AND A. DANSI, *Ann. chim. (Rome)*, 45 (1955) 31.
5 L. SANTAMARIA, *Recent Contributions to Cancer Research in Italy, Supplement to Tumori*, Casa Editrice, Ambrosiana, Milano, 1 (1960) 167.
6 A. VON GRAFFI, E. J. SCHNEIDER, H. KRIEGEL AND G. SYDOW, *Naturwissenschaften*, 40 (1953) 414.
7 A. VON GRAFFI, H. KRIEGEL, E. SCHNEIDER, G. SYDOW, V. BEYER, I. GUSE AND L. KUGLER, *Arch. Geschwulstforsch.*, 6 (1954) 226.
8 A. L. LEHNINGER AND M. SCHNEIDER, *J. Biophys. Biochem. Cytol.*, 5 (1959) 109.
9 G. M. BADGER, *Advances in Cancer Research*, 2 (1954) 73.
10 E. VON HAAM, personal communication, 1959.

Hemolysis by ultraviolet radiation

After exposure to short doses of U.V. radiation, human red blood cells undergo eventual hemolysis. Several years ago Wilbrandt *et al.*[1] gave indirect evidence that the mechanism of this hemolysis was an increase in the normally very limited permeability of the red cell to cations. As a result, the ion composition of the cells changed from the pattern seen in normal cells toward a Donnan-equilibrium state. Because of the high concentration within the cell of non-penetrating anions, chiefly hemoglobin and organic phosphates, there is a net movement of salts into the cell, accompanied by water. The cell swells and eventually hemolyzes. Wilbrandt has attempted a quantitative treatment (to a first approximation) of this process, which he termed colloid osmotic hemolysis, by treating the salts as non-electrolytes[2]. Other authors[3] have questioned this mechanism, stating that an equation is required which deals adequately with the changes in cell volume as a function of the movement of the various ions.

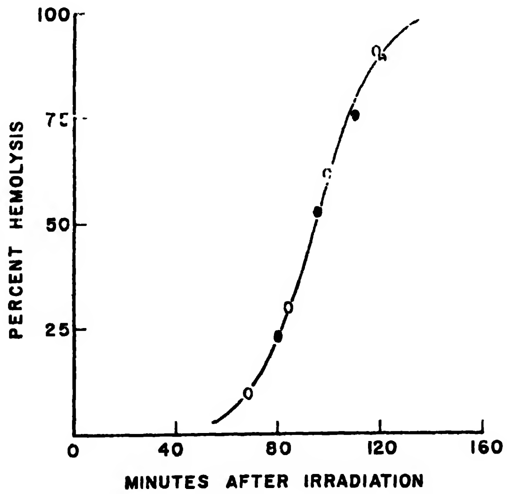

Fig. 1. Hemolysis curve following U.V.-irradiation at 254 mμ with a low pressure mercury arc. 0.3% suspension of human red cells in 0.17 M NaCl, phosphate-buffered to pH 7.5. U.V.-dose = $2.25 \cdot 10^8$ ergs incident on surface of irradiation chamber.

References p. 457

In our laboratory we have investigated this process of U.V. hemolysis with the intention of making direct measurements on ionic movements, and of deriving an equation to describe quantitatively the swelling process. After discussing this equation I shall comment on the nature of the radiation lesion in red cells.

Fig. 1 shows a typical hemolysis curve following a brief dose of U.V. radiation from a low pressure mercury arc. The slope of the straight portion of this curve is taken as a measure of the rate of hemolysis.

That there is accelerated cation movement in irradiated red cells can be seen from Fig. 2, which shows the loss of potassium from red cells suspended in buffered NaCl and irradiated at three doses. Efflux from non-irradiated cells over the time-periods used is negligible. The decrease in cell K^+ follows the first order equation:

$$\ln(K_t/K_0) = -kt \tag{1}$$

where t represents the time after irradiation, K_0 the initial cell potassium concentration, and k the rate constant for K loss. The relationship between k and the U.V. dose is plotted in Fig. 3. This log-log plot has a slope of 2; that is, the rate constant for K

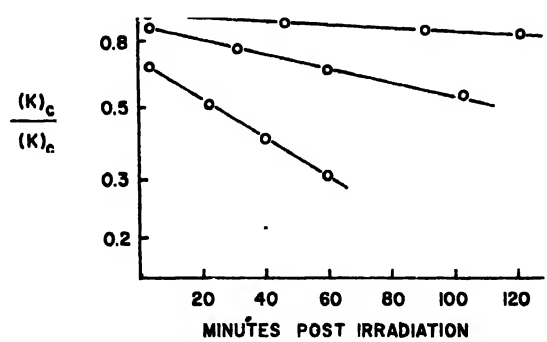

Fig. 2. Loss of potassium from U.V.-irradiated human red cells. 2% suspension of cells in 0.17 M NaCl, phosphate-buffered to pH 7.4. The upper curve corresponds to a dose of $4.5 \cdot 10^8$ ergs incident on the surface of the irradiation chamber, the middle curve is $9.0 \cdot 10^8$ ergs, the lower curve $13.5 \cdot 10^8$ ergs.

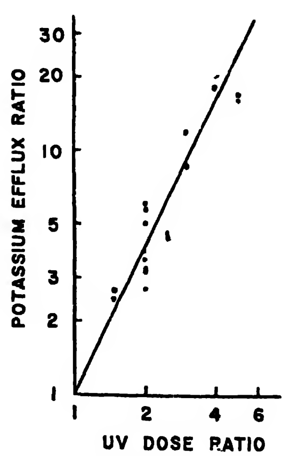

Fig. 3. Rate of loss of potassium as a function of dose. Log of ratio of rate constants, as determined from curves like those in Fig. 2., are plotted as a function of log of U.V.-dose ratio. The line has a slope of 2.

loss increases as the square of the dose. If the hemolysis process is determined by ion movements, then the rate of hemolysis should also be proportional to the square of the dose. That this is true is shown in Fig. 4, where each point represents the ratio of hemolysis rates at two doses, plotted as a function of the dose-ratio. The slope of 2 on this log–log plot means that the hemolysis rate is directly proportional to the potassium efflux.

The rate of swelling of red blood cells may be described by the equation:

$$dV/dt = \frac{1}{C}\{{}^iM - {}^oM\} \tag{2}$$

where ${}^iM$ and ${}^oM$ are, respectively, the influx and efflux of osmotically active solutes, and C is the osmolar concentration of the medium. This proportionality term implies that water moves so rapidly across the cell membrane that the cell is in osmotic equilibrium throughout. Hence, from the equation, the cell swells when the influx of solute exceeds the efflux, and shrinks when efflux exceeds influx. The flux of a given solute is given by

$$M = k_s [S] \tag{3}$$

where k_s is the rate constant and $[S]$ the concentration in the compartment from which the solute is moving.

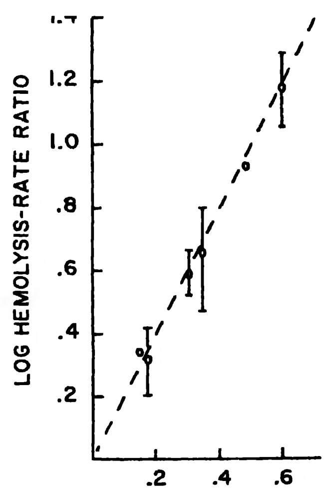

Fig. 4. Rate of hemolysis as a function of dose. Log of ratio of hemolysis rates plotted as a function of log of dose ratios. The line has a slope of 2.

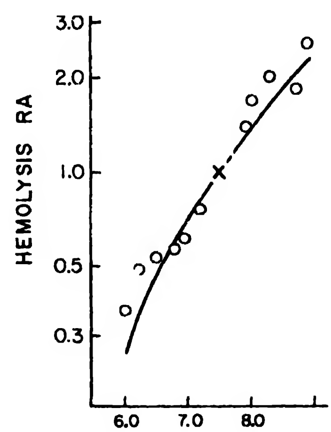

Fig. 5. Relative hemolysis rates as a function of pH. Conditions as in Fig. 1; only the pH is altered. The line is calculated from equation (6), with appropriate substitution for the various chloride ratios, ion concentrations, and initial cell volumes at the various pH. The experimental points have been normalized to the curve at pH 7.5 (X).

We have found, in experiments not described here, that the rate constant for Na flux, k_{Na}, through an irradiated red cell membrane is about 0.67 times the rate constant

References p. 457

for K flux through the same membrane, regardless of the absolute magnitude of these fluxes. This relationship may be expressed

$$\alpha = k_{Na}/k_K = 0.67 \tag{4}$$

Since we are dealing with electrolytes, allowance must be made for the driving force of the electrical potential across the membrane[4]. For red blood cells, this potential may be described[5]

$$E_m = \frac{RT}{F} \ln \frac{[Cl]_c}{[Cl]_m} \tag{5}$$

Our final assumption is that chloride moves with the cations so as to maintain electrical neutrality. The equations above may be combined and simplified to give

$$dV/dt = \frac{k_K}{C} \left\{([K]_m + \alpha[Na]_m) - \frac{[Cl]_c}{[Cl]_m} ([K]_c + \alpha[Na]_c)\right\} \tag{6}$$

where subscripts m and c represent medium and cells respectively. k_K is evaluated from the potassium efflux as shown in Fig. 2. The chloride ratio is the term correcting for the effect of the membrane potential on the corresponding influx of sodium and potassium.

Our direct measurements of dV/dt are within 30% of the values calculated from k_K and the various ion concentrations. Evaluation of this equation requires the subtraction of intracellular cation concentration from extracellular cation concentration, and these values are very similar. A 30% error in the calculated dV/dt could arise from a 2% error in measurement of ion concentrations. Consequently, we feel that the measured values are in reasonable agreement with those predicted.

A second test of the equation is possible by taking advantage of the pH effect on red cells. At low pH, the net number of negative charges on the non-diffusing anions is decreased, the chloride concentration of the cell increases, and the cell swells slightly. Because of this initial swelling, it may be expected that the cells are osmotically more fragile at the lower pH and that they would therefore hemolyze more rapidly. On the other hand, the membrane potential, as determined by the chloride ratio, is altered so as to accelerate the efflux of cations and, consequently, inhibit swelling after irradiation. Of these two factors, the membrane potential is dominant, as shown by the calculated line in Fig. 5. The experimental points show the rate of hemolysis at various pH; the line is calculated from equation (6), taking into account the altered ionic concentrations and cell volumes at the outset of the experiment. The points and the curve have been normalized at pH 7.5.

The relationships of the ionic fluxes in irradiated cells leads to a few speculations about the nature of the radiation lesion. The red cell membrane has been regarded as a permselective membrane[6], with pores lined with positive charges. These positive charges would readily permit the passage of small anions while restricting the passage of cations. If the effect of the radiation were to alter charges and thereby permit increased cation fluxes, a concomitant decrease in anion permeability would be expected. Experiments designed to show such a decrease have been consistently negative.

The rate constants of Na and K fluxes in irradiated cells are always in the ratio of

about 0.67. Since this is the ratio of the mobilities of these ions in aqueous solutions, the implication is that the radiation in some way creates aqueous channels for these ions to pass. The dose squared relationship indicates a two-hit phenomenon in the formation of such channels. The nature of this primary photochemical event remains to be determined.

ACKNOWLEDGEMENT

I am indebted to Dr. D. C. Tosteson for helpful discussion on the kinetics of cation movement.

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1 J. LEU, W. WILBRANDT AND A. LIECHTI, *Strahlentherapie*, 71 (1942) 457.
2 W. WILBRANDT, *Helv. Physiol. et Pharmacol. Acta*, 12 (1954) 184.
3 E. PONDER, *Protoplasmatologia*, 10 (2) (1955) 1.
4 H. H. USSING, *Active Transport and Secretion*, Academic Press, New York, 1954, p. 407.
5 I. M. GLYNN, *Progress in Biophysics and Biophysical Chemistry VIII*, Pergamon, London, 1957, p. 241.
6 A. K. SOLOMON, *J. Gen. Physiol.*, 43, No. 5, Pt. 2, (1960) 1.

On the apparent synergistic effect of far-red and X rays in the production of chromatid aberrations

One of the anomalous observations in photobiology has been that although infrared, or more properly far-red, radiation cannot in itself cause chromosome breakage, when it is administered with X rays it potentiates the X-ray-induced aberration yield. This had been first noticed for intergenic mutations and chromosomal aberrations in *Drosophila* by Kaufmann, Hollaender and Gay[1] and later extended to *Tradescantia* by Swanson and his coworkers[2-4]. It was thought that far red brought about either an increase in the X-ray-induced breakage[1, 2] or, conversely, decreased the restitution of the primary X-ray breaks[5]. Either process, of course, would be expected to increase the visible aberration yield.

Experiments by Moh and Withrow[6, 7] on roots of *Vicia faba* have established that, in that organism, the effective wave lengths for potentiation of X-ray-induced chromatid aberrations are around 760–780 mμ and that the far-red effect is red (wave-length 620–700 mμ) reversible. Since Gordon and Surrey[8] had found that ATP synthesis could be decreased by far red, Withrow and Moh favored the interpretation that restitution was affected, presumably by decreasing the amount of ATP, which we[9-10] had found to be necessary for the syntheses involved when breaks rejoin.

The present experiments, on the lateral roots of *Vicia faba*, were undertaken to elucidate the mechanism of the far-red-induced increase in genetic damage caused by X radiation. It was found that, in this organism at least, the increase pertained only to chromatid aberrations induced after the chromosome reacts to X rays as though it were double and not to chromosome aberrations that are formed when the chromosome reacts as though it were single. Furthermore, postirradiation far-red treatment only

References p. 460

seems to delay mitosis so that, at any given time after X irradiation, different cells are at metaphase and are sampled. The apparent increases caused by far red are really a reflection of this delay of cells in entering division so that more sensitive cells are at metaphase after a far-red treatment.

METHODS

Seeds of *Vicia faba* were soaked for 24 h, peeled, and germinated according to the method of Gray and Scholes[11]. They were then suspended with the primary roots in constantly aerated distilled water at about 20° until lateral roots were formed. The beans were then given 150 r of 250-kvp X rays at 100 r.p.m., hvl = 0.45 mm of Cu. Immediately after X irradiation, they were inmersed in water and placed in a light-tight box that had a Corning filter No. 7–69 for a top. This filter transmits light from ca. 700–1100 mμ with a rather sharp peak at 800 mμ. The box was then placed under a bank of incandescent lamps for 3 h (at the end of this time the temperature of the roots was ca. 24°). The intensity of far red as measured with a thermopile was ca. 37,000 μW/mm^2/sec. The beans were then resuspended in the aerated distilled water and samples picked every 3 h after irradiation. The roots were placed in colchicine for 2 h and then fixed in Ford's modification of Flemming fluid. Feulgen-stained smear preparations were made and 300 metaphase figures analyzed for each point.

RESULTS

In Table I may be seen the results of a typical experiment for roots picked at 24 h when almost all the aberrations are of the chromatid type and at 48 h when they are all of the chromosome type. The results at 24 h are similar to those observed by Swanson in *Tradescantia* in that there is very little increase in chromatid deletions and isochromatid breaks but a marked increase in two-hit exchanges. The total number of

TABLE I

EFFECT OF FAR RED ON RADIATION-INDUCED ABERRATIONS

Type	*150 r Aberrations*		*150 r + far-red Aberrations*	
	Number	%	*Number*	%
	24-h Fixation (chromatid aberrations)			
Chromatid deletions	34	10.2	24	8.7
Isochromatid breaks	38	11.4	25	9.1
Chromatid exchanges	10	3.0	31	11.3
Total chromatid breaks	92	27.5	111	40.4
Cells with chromosome aberrations	48	14.4	26	9.5
Total cells	334		275	
	48-h Fixation (chromosome aberrations)			
Dicentrics and rings	31	10.3	22	7.3
Interstitial deletions (dots)	19	6.3	17	5.7
Terminal deletions (rods)	8	2.7	7	2.3
Cells with chromatid aberrations	1	0.3	19	6.3
Total cells	300		300	

chromatid breaks observed after the combined far-red-X-ray treatments is significantly higher than that after X rays alone. In the same beans, however, cells from roots that were picked at 48 h when only chromosome aberrations are found, show no such increase. Similarly, several other experiments on chromosome aberrations induced in soaked seeds in which the first division of the primary root was observed showed no effect of the far red on these types of aberrations.

In Fig. 1 may be seen the curves obtained by plotting the numbers of chromatid breaks observed at the various times after X irradiation when chromatid aberrations are observable. It may be noticed in the curve, that represents the response of roots that received X rays alone, that the sensitivity increases from 3 to about 9 h after irradiation. It then seems to stay constant for several hours and later drops. This demonstrated that the cells pass through a peak of sensitivity. When the roots have been treated with 3 h of far red after X radiation, the curve shows the peak of sensitivity shifted, indicating that cells in a period of given sensitivity take longer to reach metaphase, where they can be observed. Other experiments that are less complete have given indications that the 3-h far-red treatment can shift the curve by about 2¼ h showing that it is the time that the material is being irradiated and not the total energy that is important. These curves indicate that the far-red effect observed is dependent on the time after irradiation when the cells are sampled. If cells are sampled shortly after, then it would appear that far red did not increase X-ray damage but indeed did protect against it. If cells were sampled on the plateau (it is difficult to characterize this part of the curve because of an X-ray-induced mitotic inhibition that makes it arduous to obtain many cells in division at these

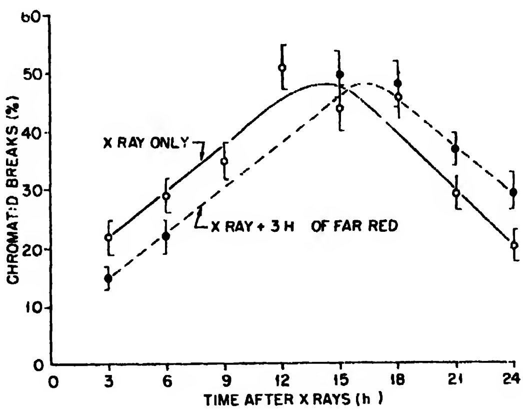

Fig. 1. Chromatid breaks at varying times after X irradiation. ○, X ray only; ●, X ray + 3 h of far red.

times), then the far red would apparently have no effect. If, however, cells are sampled as they are in most experiments, after 24 h, then there would be a seeming far-red-induced potentiation of the X-ray-induced genetic damage.

Early interphase, in which the chromosomes react to X rays as though single, is a period of uniform sensitivity to X rays. Any far-red-induced delay in mitosis therefore would not result in any apparent increase in the aberration yield.

When roots that have cells in the transitional period between the appearance of

References p. 460

chromatid and chromosome aberrations are picked, the far-red series again seems to be delayed and has fewer cells with chromosome aberrations (Table I).

CONCLUSIONS

When lateral roots of *Vicia faba* are treated with far-red radiation for 3 h after X rays, an apparent potentiation of X-ray-induced genetic damage as measured by chromatid aberrations can be observed. This seems not to be caused by an increase in chromosome breakage or by a decrease in restitution of breaks, but merely by a far-red-induced delay of mitosis. Thus, at any given time after X-ray treatment, different cells will be sampled if far red is also administered. This can result in a decrease, no effect, or an increase in chromatid aberrations after far-red treatment, depending on the time at which the cells are sampled. Chromosome aberrations that are induced when cells are in a period of uniform sensitivity show no such far-red effects.

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1 B. P. KAUFMAN, A. HOLLAENDER AND H. GAY, *Genetics*, 31 (1946) 349.
2 C. P. SWANSON AND A. HOLLAENDER, *Proc. Natl. Acad. Sci. U.S.*, 32 (1946) 295.
3 C. P. SWANSON, *Proc. Natl. Acad. Sci. U.S.*, 35 (1949) 237.
4 C. P. SWANSON AND H. T. YOST JR., *Proc. Natl. Acad. Sci. U.S.*, 37 (1951) 796.
5 B. P. KAUFMANN, *Genetics*, 31 (1946) 449.
6 C. C. MOH AND R. B. WITHROW, *Radiation Research*, 10 (1959) 13.
7 C. C. MOH AND R. B. WITHROW, *Radiation Research*, 11 (1959) 18.
8 S. A. GORDON AND K. SURREY, *Radiation Research*, 12 (1960) 325.
9 S. WOLFF AND H. E. LUIPPOLD, *Science*, 122 (1955) 231.
10 S. WOLFF, *Am. Naturalist*, 94 (1960) 85.
11 L. H. GRAY AND M. SCHOLES, *Brit. J. Radiol.*, 24 (1951) 348.

Luminescence d'hydrocarbures aromatiques. Applications à la biologie

La détection et le dosage des hydrocarbures aromatiques par fluorescence à basse température possèdent des qualités exceptionnelles de sensibilité, de spécificité et de sélectivité, qui permettent d'envisager des applications intéressantes à la biologie.

Nous considérerons le cas du 3,4-benzopyrène (3,4-BP) que nous avons étudié en détail à l'occasion d'analyses de fumées de cigarettes.

ANALYSE QUALITATIVE

Le spectre de fluorescence à 20° du 3,4-BP présente 6 bandes assez fines et intenses qui ont permis à de nombreux auteurs de le détecter dans des mélanges complexes tels que les goudrons, après traitement chimique et chromatographique de ces derniers (Fig. 1). A —180°, le spectre se résoud en bandes fines et nombreuses. Cette résolution est particulièrement remarquable pour les solutions dans les hydrocarbures paraffiniques normaux[1]. Sur la Fig. 2 est représenté le spectre à —190° d'une solution dans

* Operated by Union Carbide Corporation for the United States Atomic Energy Commission.

l'octane normal de 3,4-BP, obtenu avec un appareillage déjà décrit[2]. Plus de 60 bandes sont enregistrées.

Ce spectre très caractéristique permet des identifications quasi certaines dans des mélanges dont la grande complexité empêche d'isoler le 3,4-BP à l'état pur[3]. La Fig. 2

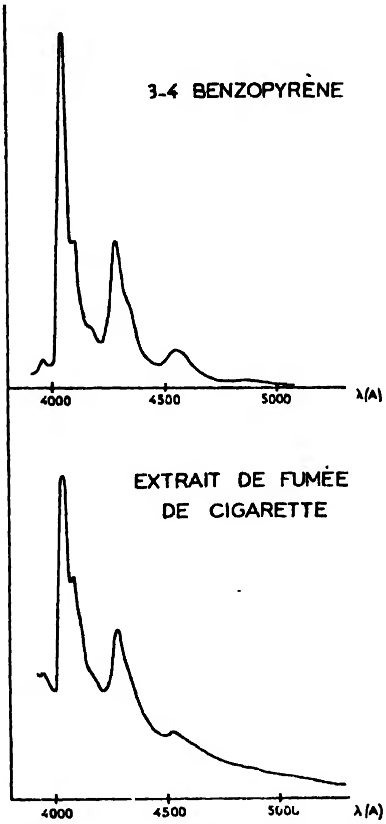

Fig. 1. Spectres de fluorescence à 20° de solutions dans des mélanges d'environ 70% de cyclohexane et de 30% de benzène, de 3,4-benzopyrène à la concentration de 1 μg/cm^3 et d'un extrait de fumée de cigarette, obtenu par traitement chimique et chromatographique. En ordonnée: flux d'énergie par unité de longueur d'onde.

en donne un exemple: les courbes B représentent le spectre d'une fraction obtenue à partir de fumée de cigarette par traitement chimique et chromatographique. Ce spectre présente 30 bandes en étroite coincidence avec celles du 3,4-BP. A la température du laboratoire, la même fraction donne un spectre moins caractéristique, bien que déjà assez significatif (Fig. 1).

La finesse et l'intensité des bandes à basse température permet de détecter le 3,4-BP au sein d'une plus grande proportion de mélanges complexes qu'à la température ordinaire. Ceci permet de diminuer le nombre des opérations de fractionnement préalables à la détection.

La sensibilité de cette technique dépasse de 10 à 100 fois celle de la technique classique. 10^{-4} μg de 3,4-BP/cm^3 sont facilement détectés et 10^{-5} μg par cm^3 donnent encore un pic décelable à 4030 Å. Cette sensibilité est telle qu'il faut purifier très soigneusement les solvants utilisés et prendre de grandes précautions pour éviter les contaminations accidentelles par des traces de 3,4-BP.

Bibliographie p. 464

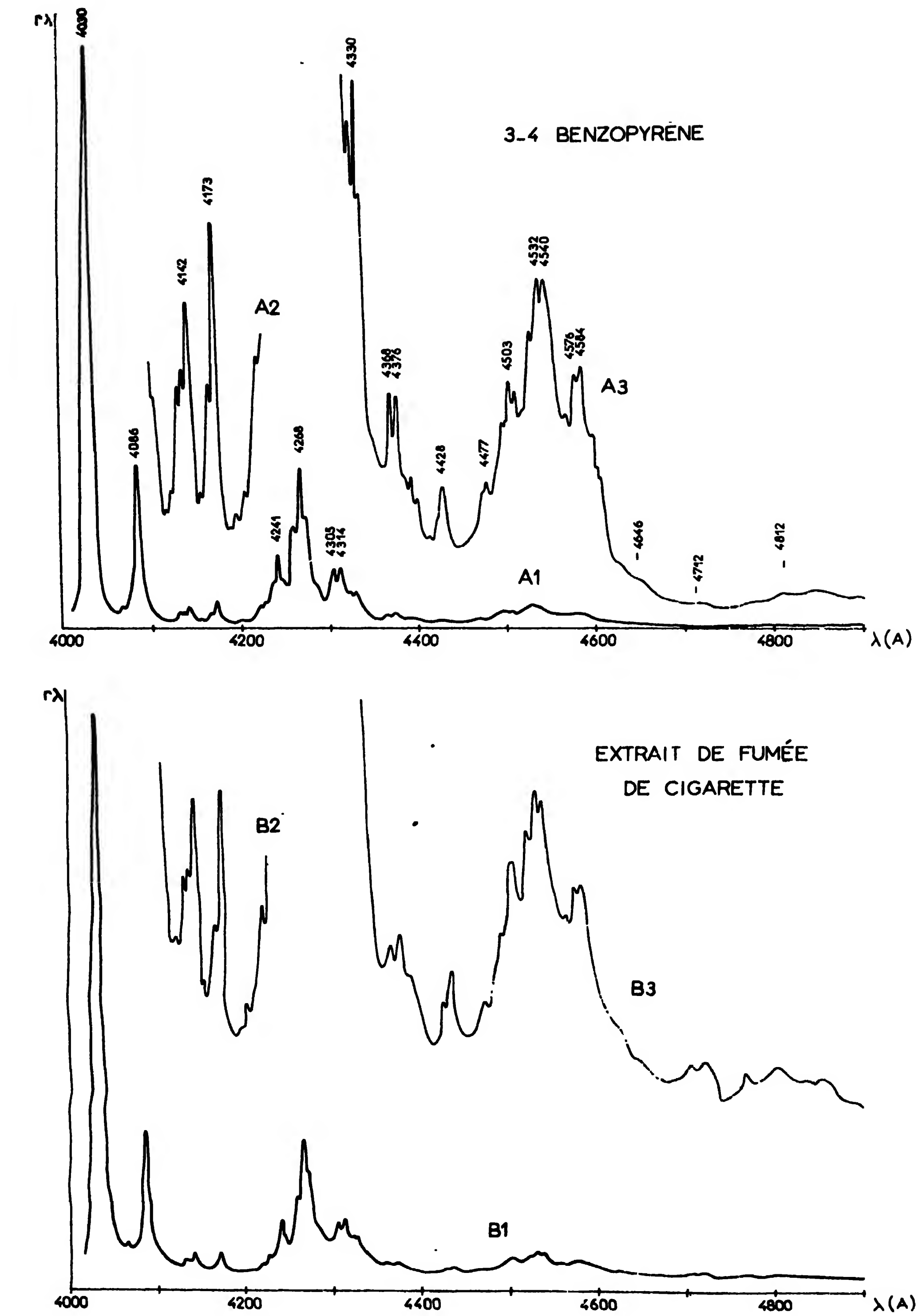

Fig. 2. Spectres de fluorescence à —190° de solutions dans l'octane. Courbes A: 3,4-benzopyrène 1 μg/cm^3. Les ordonnées des courbes A2 et A3 sont multipliées par 15 par rapport à celles de la courbe A1. Courbes B: Extrait de fumée de cigarette dont le spectre de fluorescence à 20° est représenté sur la Fig. 1. Les ordonnées des courbes B2 et B3 sont multipliées par 13 par rapport à celles de la courbe B1. En ordonnée: Flux d'énergie émis par l'échantillon dans les bandes spectrales isolées par le monochromateur, pour des enregistrements à fentes constantes.

ANALYSE QUANTITATIVE

Notre travail a consisté principalement à rendre cette méthode de détection quantitative. En effet, les dosages par fluorescence présentent certaines difficultés qui ont empêché jusqu'à présent qu'ils soient utilisés couramment: d'une part, le rendement de fluorescence d'une substance peut être fortement influencé par la présence de substances étrangères, d'autre part, aux fortes concentrations, le flux reçu par le récepteur peut ne pas être proportionnel à la concentration. Les comparaisons avec une solution étalon donnent donc des résultats incertains.

Avec la technique que nous utilisons et qui dérive de celles utilisées par d'autres auteurs[4, 6], on a obtenu sur un exemple pratique une précision meilleure que 10%, malgré les complications qu'entraîne la basse température (non reproductibilité de l'intensité de fluorescence d'un échantillon à l'autre, congelés à partir d'une même solution).

*(a) Technique**

(1) On dilue la solution à doser de façon à réduire les influences des substances étrangères et à se placer dans une zone de dilution où les réponses sont proportionnelles à la concentration[5].

(2) On prépare les solutions suivantes:

Solution	*Solution à doser diluée*	*B cm³ de solvant contenant*	*n Octane*
I	A cm³	pas de 3,4-BP	C cm³
II	A cm³	s μg de 3,4-BP	C cm³
III	A cm³	2s μg de 3,4-BP	C cm³
IV	A cm³	4s μg de 3,4-BP	C cm³

En procédant ainsi, le 3,4-BP ajouté subira les mêmes influences des substances étrangères que celui qui s'y trouve déjà[6].

(3) On mesure, à − 190°, pour chaque solution, l'émergence du pic de fluorescence à 4030 Å au dessus de la fluorescence de fond. On fait cette mesure après avoir ajusté à 4005 Å, par un réglage de la tension d'alimentation du photomultiplicateur, la déviation du galvanomètre à la même valeur pour les 4 solutions. La fluorescence du 3,4-BP ajouté étant en général négligeable, à cette longueur d'onde, par rapport à la fluorescence des impuretés, on compense ainsi partiellement des écarts dûs aux différences dans les conditions de congélation de chaque échantillon, car on peut remarquer que, si les intensités de fluorescence ne sont pas reproductibles, les rapports des intensités aux différentes longueurs d'ondes varient peu d'une congélation à l'autre d'une même solution.

(4) On porte sur un graphique, en fonction de la surcharge de 3,4-BP, les hauteurs des pics ainsi mesurées. Les points obtenus s'alignent en général à ± 5% près. On trace la droite la plus proche de ces points. La quantité de 3,4-BP contenue dans la solution I est donnée par la valeur absolue de l'abscisse de l'intersection de cette droite avec l'axe des surcharges.

* Pour les détails de la technique de dosage voir Muel et Lacroix[9].

Bibliographie p. 464

(b) Précision

Un goudron a été débarrassé du 3,4-BP qu'il contenait, par chromatographie. On y a alors ajouté 1/20000 en poids de 3,4-BP pur. Plusieurs dosages par la méthode décrite ont donné des erreurs inférieures à 10%. La méthode, moins sûre, de simple comparaison avec une solution étalon a cependant donné la même précision sur cet exemple particulier, à condition d'utiliser des solutions extrêmement diluées, ce qui rend alors les manipulations très délicates en raison du danger des contaminations.

APPLICATIONS

(a) Fumée de cigarette

De nombreuses applications ont été faites dans ce domaine. Elles sont relatées dans la thèse de Monsieur Hubert-Habart[7]. On trouve 10^{-2} μg de 3,4-BP par cigarette.

(b) Alcools distillés destinés à la consommation

Nous avons pu caractériser d'une façon incontestable du 3,4-BP dans des échantillons d'alcools divers. Un dosage dans le kirsch a donné $5 \cdot 10^{-4}$ μg de 3,4-BP par cm^3.

(c) Eau du réseau de distribution urbain

Nous n'avons pu détecter, dans l'échantillon analysé, aucune quantité notable de 3,4-BP. On peut affirmer qu'il contenait moins de 10^{-5} μg de 3,4-BP par cm^3.

(d) Détection des métabolites liés aux protéines après badigeonnage d'hydrocarbures sur la peau de souris

Nous nous sommes demandés si la fluorescence à basse température ne pourrait pas permettre de détecter et de caractériser les très petites quantités de métabolites qui ont été trouvées, par la méthode des éléments marqués, liées aux protéines cellulaires, après badigeonnage par des hydrocarbures aromatiques de la peau de souris.

Des essais dans ce sens ont été entrepris, en collaboration avec Monsieur et Madame Daudel et sous la direction de Monsieur Latarjet. Les premiers résultats montrent que la technique de fluorescence à basse température permet d'obtenir plus d'informations que la technique classique de fluorescence à 20°[8]. Des spectres assez détaillés ont été obtenus et leur étude est en cours.

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1 E. V. CHPOLSKII ET E. A. GIRDJIIAOUSKAITE, *Optica i Spectroskopiya*, 4 (1958) 620.
2 B. MUEL ET M. HUBERT-HABART, *J. chim. phys.*, 55 (1958) 377.
3 P. P. DIKOUN, *Voproci Onkologii*, 5 (1959) 672.
4 A. A. IL'INA, *Izvest. Akad. Nauk S.S.S.R.*, Ser. Fiz., 15 (1951) 771.
5 A. KLING ET M. HEROS, *Compt. rend.*, 208 (1939) 2075.
6 E. J. BOWEN ET F. WOKES, *Fluorescence of Solutions*, Longmans, London, 1953.
7 M. HUBERT-HABART, *Thèse*, Paris, 1960.
8 D. S. TARBELL *et coll.*, *Cancer Research*, 16 (1956) 37.
9 B. MUEL ET G. LACROIX, *Bull. soc. chim. France*, (1960) 2139.

Area intensity relations in human dark-adapted vision

Studies of visual acuity with free eye movements and long exposures[1–3] have shown that the retinal region mediating the best acuity shifts from the more coarsely structured peripheral retina to the fovea as the light intensity rises. Spatial summation of light energy (*e.g.* ref.[4]) is more extensive, and the absolute light threshold lower[5], in the more peripheral parts of the visual field. If visual acuity and the absolute threshold depend on some form of spatial organization of the receptors then comparison of the two functions under the same conditions in the same eye, using corresponding test objects, is important. The method of fixation and brief (2.6 msec) flash allows study of a particular eccentricity of the visual field with maximal spatial summation[6] and constant full dark-adaptation.

Strictly, visual acuity involves the resolution of the details of a test object, but Pirenne[7] introduced the *detection* of a black disc against a briefly lit, but otherwise dark, background as a major simplification of the visual acuity task, and this is the opposite case to the detection of a brief flash against zero intensity background. Thus the visual system is kept in a state of full dark-adaptation and has to detect either a minimal amount of excitation due to a light target (absolute threshold task) or a minimal lack of excitation due to a dark target (simple scotopic acuity task).

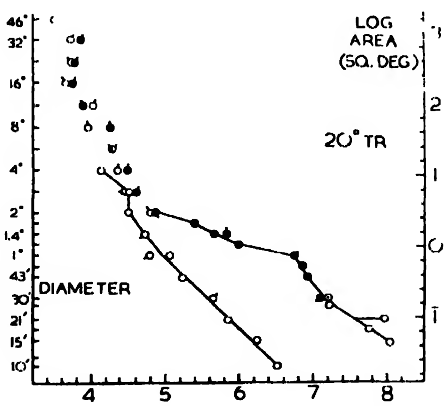

Fig. 1. Area-intensity relations at 20" temporal retina. Absolute threshold results as empty circles, o. Scotopic acuity results as filled circles, ● and ⊙

It may here be helpful to introduce a unit hypothesis[7,8] which stems from the earlier theory of Selig Hecht[9]. Hecht postulated that the receptor thresholds are distributed in a Gaussian fashion and that there is therefore recruitment of receptors, and rise in acuity, with intensity rise. The modern theory stresses instead the importance of the inevitable quantum fluctuations in the stimulus itself and the spatial summation between the effects of the absorbed quanta. Considering a given peripheral

References p. 467

region, recruitment does occur, but as probability recruitment of units, perhaps similar in threshold, and composed of a great many receptors. At very low intensities the probability of any unit absorbing its threshold number of quanta is small yet, by chance, a few of the many units do reach threshold. A black disc must be larger than the naturally occurring dark gaps between the excited units if it is to be detected at all. As flash intensity rises more units respond, the dark gaps shrink and smaller discs become visible. If a large dim field contains only a few excited units its shape cannot be properly known. Confusions between large circular and semicircular fields in the periphery do actually occur at threshold.[10]

At 20° from the fovea the scotopic acuity versus flash intensity curve is divided by a pronounced break into two principal limbs. The break (1°-1.2° disc diameter) is of the same order of size as the complete summation area for the absolute threshold (1°–2° diameter). The first, or lower, acuity limb has, for discs larger than 2° diameter, much the same area-intensity relations as the absolute threshold for large flashed fields. For discs smaller than 2° this limb deviates rapidly to higher intensities, reaching its maximum acuity at the break between the two limbs. The second, or higher, acuity limb extends from the break to the final, maximal, acuity plateau[11] and is displaced to much higher intensities than the absolute threshold intensities of the corresponding sizes of fields, *i.e.* fields for which spatial summation is complete. The second limb has a distinctive character: subjective changes in disc appearance occur over its intensity range and preliminary experiments suggest that it is less sensitive to weak light adaptation than is the first limb.

One might postulate firstly, that the complete summation area gives some measure of unit size, and secondly, that a unit cannot detect a dark object much smaller than itself. In view of the possible complexities of visual mechanisms the agreement between the complete summation area and the acuity break is remarkable. This fact suggests, together with the initial parallel between the first limb and absolute threshold and also the low intensities (one quantum absorbed per several thousand receptors) at which the first limb arises, that the absolute threshold and first acuity limb represent the probability recruitment, by increase in area and intensity respectively, of low quantum threshold units of about 1° in size. Overlap of receptive fields with sharing of receptors can scarcely affect the relation between summation, maximum acuity and unit size. There remains the possibility that a unit shadowed by a disc smaller than itself will give a nervous response significantly smaller than that of unshadowed units. The rough agreement between summation area and acuity break suggests that the contribution of such a mechanism is not very great.

So far as the second limb is concerned two possible mechanisms may be considered: suprathreshold activity of big units, the recruitment of which is supposed to produce the first limb, or probability recruitment of small units hitherto inactive because their small size limits their ability to capture a threshold number of quanta at low intensities. It is unlikely that the change from incomplete probability recruitment of big units to suprathreshold activity would be sufficiently marked to produce a distinct break of the observed size.

Previous workers have studied either the higher or the lower acuity ranges but not the region of the new break described here.

Pirenne[7] used disc test objects with diameters of more than 1°. Under slightly different conditions he noted the probable existence of a small break at 2° disc diam-

eter and was able to fit his results to curves shewing the probability, at any intensity, of the disc covering a unit that would otherwise have reached its threshold of 4–10 quanta. The present results do not fit these curves and it seems that flash duration or individual differences between subjects can exert marked effects on the slope of the first limb.

Mandelbaum and Sloan[11] used Landolt C test objects with gaps subtending angles of less than 1°. They were able to demonstrate a change from rod to cone function just before the final acuity plateau was reached.

One may conclude that there is some evidence that the recruitment of units with large summating receptive fields is one of the mechanisms of peripheral dark-adapted vision, though the interpretation of these units in terms of neurons is not yet possible.

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1 S. SHLAER, *J. Gen. Physiol.*, 21 (1937) 165.
2 M. H. PIRENNE AND E. J. DENTON, *Nature*, 170 (1952) 1039.
3 M. H. PIRENNE, F. H. C. MARRIOTT AND E. F. O'DOHERTY, *Spec. Rept. Ser. Med. Research Council*, H.M.S.O., London, 1957, p. 294.
4 C. H. GRAHAM, R. H. BROWN AND F. A. MOTE JR., *J. Exptl. Psychol.*, 24 (1939) 555.
5 W. S. STILES AND B. H. CRAWFORD, *Proc. Roy. Soc.* Ser. B, 122 (1937) 255.
6 C. H. GRAHAM AND R. MARGARIA, *Am. J. Physiol.*, 113 (1935) 299.
7 M. H. PIRENNE, *Proc. Cambridge Phil. Soc.*, 42 (1946) 78; M. H. PIRENNE, *Vision and the Eye*, Chapman and Hall, London, 1948, p. 120.
8 M. H. PIRENNE AND F. H. C. MARRIOTT, in S. KOCH (Editor), *Psychology: A Study of a Science*, Vol. I, McGraw-Hill, New York, 1959, p. 288.
9 S. HECHT, *J. Gen. Physiol.*, 11 (1928) 255.
10 M. H. PIRENNE, *J. Physiol.*, 107 (1948) 47.
11 J. MANDELBAUM AND LOUISE L. SLOAN, *Am. J. Opthalmol.*, 30 (1947) 581.

Session 3

PHOTOSYNTHESIS

Chairman: DANIEL I. ARNON, Berkeley, Calif. (U.S.A.)

Secretary: K. VEJLBY, Copenhagen (Denmark)

Comparative kinetics of the photosynthesis and whole-cell Hill reaction of *Chlorella**

INTRODUCTION

It has suggested by many workers that the photochemical production of oxygen by photosynthesis and by the Hill reaction must share common reaction pathways, at least in part. However, relatively little comparative work on the kinetics of oxygen evolution in the two processes has been published. It is difficult to make comparative measurements using leaves and isolated chloroplasts of higher plants. The best system appears to be the photosynthesis and whole-cell quinone Hill reaction[1] of unicellular algae such as *Chlorella*.

Clendenning and Ehrmantraut[2] found that higher light intensities were required to saturate the *Chlorella* Hill reaction as compared to photosynthesis of the same cells. The period of the limiting dark reaction in flashing-light measurement was about the same in each case. However, Ehrmantraut and Rabinowitch[3] showed that both the maximum yield per flash and the quantum yield were the same for the two processes, but that the shapes of the light-saturation curves under steady-state conditions were different. Whittingham[4] also observed this latter relationship.

Fraser[5] found that *Chlorella* photosynthesis was much more sensitive to iodoacetamide and iodoacetic acid than the quinone Hill reaction. Huzisige[6] examined the comparative effects of hydroxylamine, azide, cyanide, and 2,4-dinitrophenol on *Chlorella* photosynthesis, Hill reaction and "catalase activity". Hydroxylamine inhibited both photosynthesis and the Hill reaction at essentially the same concentration range. Azide inhibited both, however the "order" of the inhibition was two for photosynthesis and one for the Hill reaction. Cyanide inhibited photosynthesis but not the Hill reaction, while dinitrophenol inhibited both.

This paper presents the results of some preliminary studies carried out in our laboratory on the comparative kinetics of oxygen evolution by *Chlorella* during photosynthesis and during the whole-cell quinone Hill reaction.

MATERIALS AND METHODS

All of the experiments reported here were carried out with *Chlorella pyrenoidosa* (Emerson strain-type D). The algae were cultured under controlled conditions in a modified Knop's solution at 25° in flasks aerated with 3% carbon dioxide in air. Illumination was provided by Sylvania "cool white" fluorescent tubes. The cultures were grown under continuous light for six days followed by six cycles of 12 h each of light and dark to provide "synchronized cultures"[7].

Rate measurements of the oxygen evolved in photosynthetic and Hill reaction systems were made manometrically using rectangular vessels and a water bath equipped with General Electric 150 watt reflector-spot lamps arranged to illuminate the reaction vessels from below. All comparative measurements were made using aliquots of the same suspension of algae. Photosynthetic reaction systems contained

* This work was supported by the U.S. Atomic Energy Commission under Contract AT(11-1)-875.

References p. 472/473

2.0 ml of algae suspended in Warburg No. 9 buffer plus 1.0 ml of distilled water or inhibitor solution. Hill reaction systems consisted of 1.0 ml of algae (at twice the concentration used in photosynthetic experiments) suspended in distilled water, 1.0 ml of 0.012 *M* *p*-benzoquinone dissolved in pH 6.8 phosphate buffer, and 1.0 ml of distilled water or inhibitor solution.

RESULTS

The interactions between the effects of oxidant concentration, pH; light intensity, temperature, inhibitor concentration, etc. on the rates of oxygen evolution in algal photosynthesis and Hill reaction are exceedingly complex. Only a few examples of the experimental results can be presented in the space available.

As previously observed by others, *p*-benzoquinone was found to be a much better oxidant than ferricyanide with fresh whole cells. The Hill reaction rate at high light intensity was strongly dependent on oxidant concentration. The rate was maximal at approximately $4 \cdot 10^{-3}$ *M* quinone, and fell off sharply both above and below this concentration. The same general behavior has been observed in this laboratory with the Hill reaction of the isolated chloroplasts of higher plants. It may be that this phenomenon results from an uncoupling of the Hill reaction and photosynthetic phosphorylation by the oxidant[8], although little is known about uncoupling processes in whole cells. At high light intensity the algal Hill reaction rate was maximal at approximately pH 6.5.

At saturating light intensities, and at temperatures of 20–30°, the rates of oxygen evolution were essentially the same for photosynthesis and for the Hill reaction. The shapes of the rate *vs.* light intensity curves were quite different, however, with photosynthesis approaching saturation at much lower light intensities than the Hill reaction. The temperature coefficient for the rate-limiting dark reaction of photosynthesis (as observed at saturating light intensities) was greater than that for the Hill reaction, with the experimental activation energies being about 11 kcal for the former process and 8 kcal for the latter. The quinone Hill reaction system was rapidly and irreversibly inactivated at temperatures of 30° and higher, (while photosynthesis could be measured up to 40°.

The herbicide CMU (*p*-chlorophenyl-1,1-dimethylurea) was a potent inhibitor of both photosynthesis and the Hill reaction of *Chlorella*, although the interactions of CMU concentration, light intensity and temperature on reaction rates were quite different in the two reactions. CMU inhibited photosynthesis and the Hill reaction at both high and low light intensities; the inhibitory effect was greater at low intensities. The general shape of the light saturation curves for photosynthesis was the same for both control and CMU-inhibited algae. In the case of the Hill reaction, however, CMU inhibition was so much greater at low light intensities that the curves for inhibited algae were sigmoid rather than hyperbolic. CMU inhibited both photosynthesis and the Hill reaction completely at concentrations much lower than the "average" chlorophyll concentration in the reaction system.

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[1] O. WARBURG AND W. LÜTTGENS, *Biokhimiya*, 11 (1950) 303.
[2] K. A. CLENDENNING AND H. C. EHRMANTRAUT, *Arch. Biochem.*, 29 (1950) 387.

3 H. EHRMANTRAUT AND E. RABINOWITCH, *Arch. Biochem. Biophys.*, 38 (1952) 67.
4 C. P. WHITTINGHAM, *Research in Photosynthesis* (ed. H. GAFFRON), Natl. Acad. Sci., Natl. Research Council, New York, 1957, p. 263.
5 I. M. FRASER, *Australian J. Exptl. Biol. Med. Sci.*, 32 (1954) 49.
6 H. HUZISIGE, *J. Japan. Biochem. Soc.*, 41 (1954) 605.
7 H. TAMIYA, S. MIYACHI AND T. HIROKAWA, *Research in Photosynthesis* (ed. H. GAFFRON), Natl. Acad. Sci., Natl. Research Council, New York, 1957, p. 213.
A. T. JAGENDORF, *Federation Proc.*, 18 (1959) 974.

Transient fluorescence changes in chloroplasts during the photochemical bleaching of chlorophyll *in vivo*

The intense exciting light required foi observation of chlorophyll distribution *in vivo* by fluorescence microscopy, causes, during photochemical bleaching, some striking changes in the spectral character of emission. These changes, which do not occur in oxygen deficient cells, involve the development of a bright yellow fluorescence following a rapid decay of the normal red fluorescence. Oxygen deficient cells remain unbleached and appear unimpaired by the intense light through continued normal protoplasmic streaming or flagellar motion in appropriate species. Aerated cells, on the other hand, show irreversible effects of injury after development of yellow fluorescence. That the spectral changes are confined to chlorophyll sites within the chloroplast is confirmed by comparison, through photography, of the red and yellow emission images and the chlorophyll absorption image at 4358 Å.

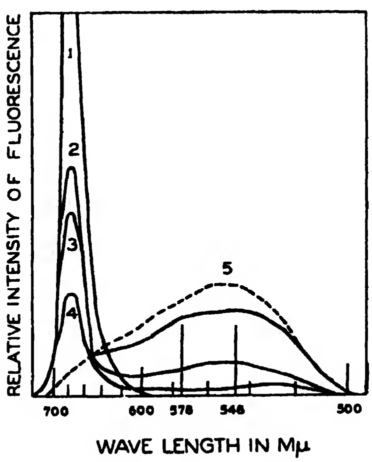

Fig. 1. Sequence of typical changes in the emission spectrum of chloroplasts during photochemical bleaching.

In an earlier report of the foregoing effects[1], we included spectral emission curves measured by means of a rapid scanning microspectrofluorimeter designed for the purpose[2]. The intense exciting light (2–3 W/cm² on the specimen) produced by isolating the 4358 Å region from a 200 W mercury arc was excluded from emission by suitable cutoff filters transmitting down to 4850 Å. In order to prevent uncontrolled oxygen deficiency, specimens of *Elodea* were perfused while those of *Chlorella* and *Euglena* were suspended in liquid films or droplets.

References p. 476

Typical examples of data are given in Figs. 1–3. Fig. 1 shows the general character of spectral changes in emission during bleaching in an airsaturated medium. Fig. 2 shows the time course of emission at the selected wavelengths of 680, 630, and 578 mμ along with that of relative transmission at 435 mμ. The red fluorescence peak shows a rapid initial decrease during the induction period of bleaching and the development of yellow fluorescence. (Prolonged light exposure attenuates the yellow fluorescence).

Following a decay of the red peak to nearly 10% of the original level, almost complete recovery can occur during several minutes of dark. Recovery from greater decay is inversely proportional to the degree of bleaching and yellow fluorescence developed. Reversal of bleaching and yellow fluorescence in the dark is rarely observed.

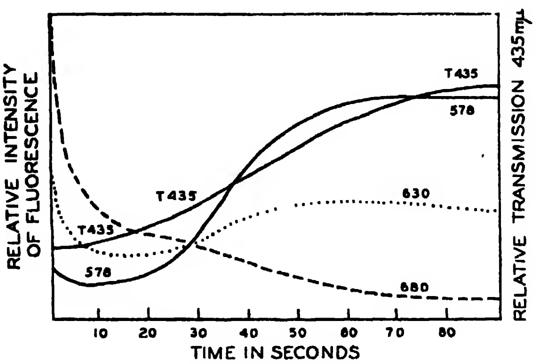

Fig. 2. Time course of emission at selected wave lengths, along with relative transmission.

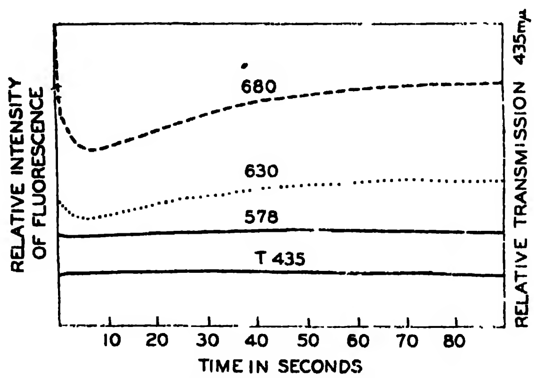

Fig. 3. Same as Fig. 2 but in the absence of oxygen.

In the absence of oxygen (Fig. 3) no bleaching or yellow fluorescence develops and the red fluorescence, after recovery from an initial slight decay, remains unchanged.

All of the effects shown in Figs. 1–3 can occur in the presence of numerous narcotics and poisons, and in the intact chloroplasts of cells which have been immersed in boiling water. They do not appear, therefore, to depend on the cellular integrity required for normal metabolism. In preparations which dry out during observation, however, spectral changes cease and fluorescence disappears.

The occurrence of spectral changes as a primary effect of heat injury is unlikely in view of the lack of injury in oxygen deficient cells where energy absorbance remains

at maximum*. Furthermore, spectral changes are not diminished in preparations cooled by rapid perfusion.

Energy in the region of the red absorption of chlorophyll (6780 Å) also produces the changes caused by the 4358 Å region. This eliminates the possibility of the specific action of spurious ultraviolet energy and also limits the pigments directly involved to those absorbing in the red region.

Recent preliminary findings bear on the identification of the yellow fluorescent material. Few known native chloroplast constituents conform to requisite properties of absorption and emission. The yellow fluorescence observed in total pigment extracts is very weak and that observed in chlorophyils *a* and *b* is hardly measurable. The frequent observation, however, of more intense yellow fluorescence occurring on foreign

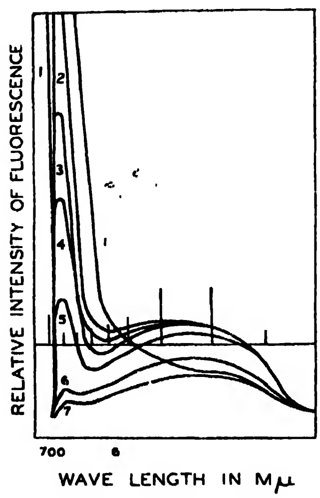

Fig. 4. Sequence of typical changes in the emission spectrum of chlorophyll *a* adsorbed on silica gel.

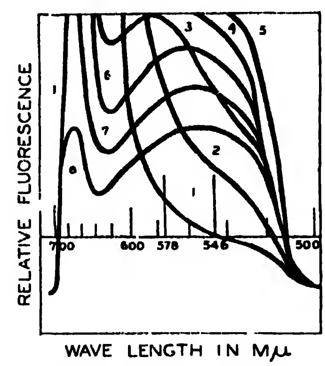

Fig. 5 Same as Fig. 1 with intensity scal expa: d·d.

particles in extracts suggested that adsorption on surfaces might enhance the effect. Silica gel was chosen as an ideal adsorbent on the basis of earlier quenching studies[3] and more recent photobleaching experiments[4] using chlorophyll adsorbed on that material. Accordingly, crystalline purified chlorophyll *a***[5] was dissolved in pyridine and adsorbed on activated silica gel. Under similar optical conditions employed in the observation of chloroplasts, the gel particles (mean diameter 150 μ) show initially a bright red fluorescence. This is replaced rapidly in air by a bright yellow emission similar to that of chloroplasts (Figs. 4 and 5). Co.·inued excitation leads to gradual disappearance leaving the very weak greenish background observed with pyridine alone on silica gel. Recovery and bleaching is similar to that in chloroplasts (bleaching is more difficult to observe due to the very thin layer of adsorbed chlorophyll).

The similarity of photochemical events in chloroplasts to those occurring in chlo-

* Since the 1-μ region of the source is excluded by copper sulfate solution, energy absorbed at 4358 Å by suspended cells is free to escape to the surrounding, nonabsorbing aqueous medium.
** From samples kindly provided by Drs. Trurnit and Colmano of R.I.A.S., Baltimore, Md., U.S.A.

References p. 476

rophyll aggregates on silica gel is significant with respect to the possible functional role of pigment surfaces of monolayers in the chloroplast. Evstigneev and Gavrilova[6] have stressed this role in a study of photosensitization by colloidal aggregates of chlorophyll. The formation of an intense yellow fluorescent material in purified chlorophyll *a* preparations *via* spectral changes nearly identical to those observed in chloroplasts, confines the reactants involved to those occurring in such purified extracts. In view of no known qualifying native impurity as a reactant, and, in view of the profound changes in the optical properties of chlorophyll that occur, it is suggested that chlorophyll in intense light combines with oxygen to form an accumulative end product with a fluorescence near 560–570 mμ. (In support of the formation of a labile peroxide is the physical injury effect observed in living cells during the course of development of yellow fluorescence.) Since these results are preliminary, and serve mainly to identify the yellow emission, an adequate hypothesis to account for the sequence of events and the physical state of the pigment complexes involved awaits further investigation. The study of such a system, however, should add to the present knowledge of reactions involved in bleaching and other photochemical properties of chlorophyll in chloroplasts.

ACKNOWLEDGEMENTS

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1 R. A. OLSON, C. L. GREENBLATT AND E. K. ENGEL, *Biophysical Society Meeting (Pittsburgh, Pa., 1959)* (to be published).
2 R. A. OLSON, *Rev. Sci. Instr.*, 3 (1960) 844.
3 H. KAUTSKY AND A. HIRSCH, *Ber. deut. chem. Ges.*, 64 (1931) 2677.
4 K. HAYASHIYA, Z. YASHIDA, R. ODA AND Y. HAMAMURA, *J. Agr. Chem. Soc. Japan*, 29 (1955) 45.
5 H. TRURNIT AND G. COLMANO, *Science*, 128 (1958) 478.
6 V. B. EVSTIGNEEV AND V. A. GAVRILOVA, *Doklady Akad. Nauk S.S.S.R.*, 126 (1959) 410.

Carotenoid protection of porphyrins from photodestruction*

Recently there have been a number of investigations with unicellular organisms illustrating that carotenoids protect against cell death or chlorophyll destruction[1,2]. Koski and Smith[3] have reported that an albino mutant in corn, white-3, forms protochlorophyll and chlorophyll. However, the chlorophyll was destroyed upon continued illumination.

This report summarizes our studies with this so-called albino mutant. When the mutant seedlings are grown in the greenhouse they contain no signs of colored carotenoids, but, instead, they accumulate large amounts of phytoene, a precursor of colored carotenoids which has absorption maxima in hexane[4] of 275, 285, and 297 mμ. The experiments on photosensitivity of chlorophyll and catalase of mutant and normal seedlings were made on seedlings which had been germinated and grown in the dark for 7-10 days. Each determination consisted of 6 whole plants which were placed in 250 ml suction flasks. The flasks were twice evacuated in the dark and each time re-filled with either air or nitrogen. The plants in the flasks were illuminated with 1500 ft.-c. of light produced by a bank of tungsten filament lamps. After the exposure period, leaves of the plants were removed, weighed and immediately ground using a mortar and pestle, with 80% acetone and a little sand. This was for chlorophyll determination. Catalase was measured by the method of permanganate[5] titration of H_2O_2.

As reported by Koski and Smith[3], dark-grown mutant seedlings contained as much and usually more protochlorophyll than did normal seedlings. The protochlorophyll of both types of seedlings was readily converted to chlorophyll upon exposure of the

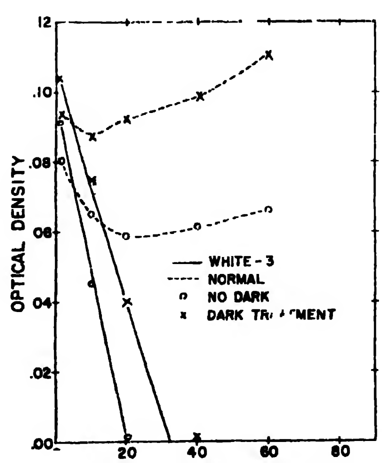

Fig. 1. Photostability of chlorophyll in white-3 and normal seedlings.

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References p. 479

seedlings to light. The results of exposing mutant and normal seedlings to light under various conditions are reported in Fig. 1. Chlorophyll in leaves of the mutant was completely destroyed when seedlings were illuminated in an atmosphere of air. Under anaerobic conditions, however, chlorophyll in the mutant was stable to high light intensity. When leaves in normal seedlings were illuminated in air, there was an initial decrease in chlorophyll content for the first 20 min followed by a stabilization and eventually an increase in chlorophyll content. It was suspected that this phenomenon might parallel the conversion of chlorophyllide *a* to chlorophyll *a*. Studies showed that most of the pigment which was formed after one minute of light by the normal plant seedlings was chlorophyllide, and in a subsequent longer dark period, it was esterified to form chlorophyll. Seedlings treated so as to contain mainly chlorophyllide or chlorophyll *a* were exposed to light and the stability of chlorophyll measured. It was found that chlorophyllide was more unstable than was chlorophyll *a*.

Catalase of the carotenoidless mutant was also photosensitive. Under aerobic conditions catalase activity of mutant seedlings slowly declined during 8 h of light to one-third of the initial value. Catalase activity of normal seedlings was not affected by this treatment. Again, under anaerobic conditions, catalase of the mutant was stable to light.

White-3 seedlings germinated and grown under dim light (0.5 ft.-c.) become blue-green. Carotenoid pigment analysis shows that the seedlings contained phytoene, phytofluene, and ζ-carotene in the following amounts: 120, 32, and 17 μg per g of fresh weight, respectively, and 30 μg of chlorophyll. Chlorophyll, ζ-carotene, and phytofluene, are all destroyed within 15 min when these seedlings are exposed to bright sunlight. The rate of destruction of the three pigments appears to be similar. Phytoene is not destroyed by sunlight. This is probably due to the fact that only trace amounts of light of the wave lengths absorbed by phytoene come through the earth's atmosphere. Protein in the seedlings also would screen out light of this quality and thereby protect phytoene.

The above results indicate that ζ-carotene, phytofluene and photoene of this mutant are not capable of protecting chlorophyll from photodestruction. One must realize that these carotenes are comparatively highly saturated and are present only in trace amounts. With respect to this phenomenon, we[6] have found that a temperature sensitive mutant of corn, which under high temperature contains one-tenth the normal amount of β-carotene, is not able to protect against the destruction of approximately two-thirds of its chlorophyll when illuminated with as little as 115 ft.-c. of light.

Leaves of the mutant and normal seedlings were imbedded in paraffin and sectioned. The chloroplasts of the mutant appeared to be of the same size and to be as numerous as those on normal seedlings. A green colored pellet containing chloroplasts could be sedimented from a buffered isotonic extract of the mutant. These chloroplasts were capable of carrying out the Hill reaction as measured by ferricyanide reduction[7].

In summary, there is a metabolic block in carotene biosynthesis in white-3. The block allows the formation of some ζ-carotene but this carotene, or the amount of it formed, is not able to protect chlorophyll from photodestruction. Chlorophyll and chloroplast formation by the mutant appears to be normal, but its chlorophyll and catalase are destroyed by high light intensities under aerobic conditions. Under anaerobic conditions, however, these porphyrins of the mutant are stable to strong

light. The results support the thesis that an important role of colored carotenoids is the protection of chlorophyll and other porphyrins from photodestruction.

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1 M. DWORKIN, *Nature*, 184 (1959) 1891.
2 M. MATHEWS AND W. R. SISTROM, *Nature*, 184 (1959) 1892.
3 V. M. KOSKI AND J. H. C. SMITH, *Arch. Biochem. Biophys.*, 31 (1951) 180.
4 I. C. ANDERSON AND D. S. ROBERTSON, *Plant Physiol.*, 35 (1960) 531.
5 B. CHANCE AND A. C. MAEHLY, *Methods of Enzymology*, Vol. 2, Ed. S. P. COLOWICK AND N. O. KAPLAN, Academic Press, New York, 1955, p. 764.
6 D. S. ROBERTSON AND I. C. ANDERSON, *J. Heredity*, 52 (1961) March April issue.
7 D. W. KROGMANN AND A. T. JAGENDORF, *Plant Physiol.*, 32 (1957) 373.

Is the induction phenomenon of *Chlorella* fundamentally different from that of most other plants?

One of us has previously investigated[1-5] the photosynthesis CO_2-time curves of different species of plants, mainly mosses, by means of the gas thermal conductivity method. Most of these time-curves showed an induction peak, *i.e.* a peak corresponding to illumination of about half a minute. A small number of species did not react in this way but had gradually increasing time-curves throughout the first few minutes of illumination.

A third type of CO_2-time curve was found by Emerson and Lewis[6] in experiments with *Chlorella pyrenoidosa*. Here the reaction upon illumination was neither a sudden short uptake of carbon dioxide (an induction peak), nor a gentle slope of the time-curve, but a burst or gush of carbon dioxide. This discovery has been confirmed by several investigators among whom van der Veen[7] further demonstrated a similar reaction on sudden illumination for another green algae, *Protococcus olivaceus*. The carbon dioxide burst from *Chlorella* has been investigated in further detail by Brown and Whittingham[8], who also observed the same reaction in a third species of green algae, *Scenedesmus obliquus*, and by Hiller and Whittingham[9], who demonstrated that only certain strains of *Chlorella pyrenoidosa* give off carbon dioxide on illumination.

In the *Chlorella* investigations mentioned above, the plant material was cultivated in a liquid medium through which bubbled carbon dioxide-enriched air (*ca.* 4–5% CO_2). The measurements of the photosynthesis were made in a similar CO_2-concentration. As far as the authors are aware only two exceptions to these experimental conditions have been reported: *McAlister* and *Myers*' investigations[10] of 1940 and *Gaffron*'s report[11] of 1957. In the first case the *Chlorella* was grown in flasks through which bubbled either air or air containing 4% CO_2, but the photosynthesis was studied in air containing 0.03 to 0.33% CO_2. In the second case "air stream grown *Chlorella*"

References p. 481

was used. The photosynthesis–time curves obtained under these conditions did not show any sign of a CO_2 burst at the commencement of illumination.

We decided to investigate more thoroughly the influence of carbon dioxide concentration on the CO_2-gush in *Chlorella*. In our first experiments we used algae cultivated in a liquid aerated with 5% CO_2. On measuring their time-curves in air containing 3% CO_2 we found the familiar burst of carbon dioxide immediately following the commencement of illumination (Fig. 1A). However, using algae from the same culture and measuring the photosynthesis in atmospheric air containing only 0.03% CO_2, we obtained quite different results (Fig. 1B). The time-curve increased gradually throughout the whole illumination period; no CO_2-gush appeared and there was no sign of a peak.

In a previous investigation using moss plants it had been found to be an experimental advantage to measure the time-curves in alternating light and dark periods[2]. The *Chlorella* experiments were conducted on similar lines (Fig. 2C). Here again the

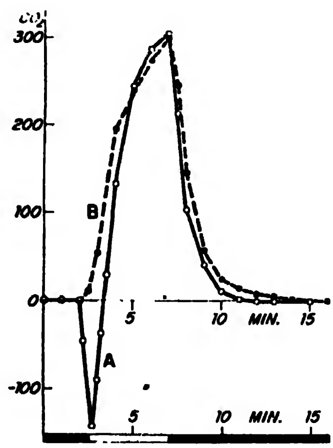

Fig. 1. Photosynthesis–time curves for *Chlorella pyrenoidosa* cultivated in a liquid medium through which air enriched with 5% CO_2 was bubbled. The measurements were made in atm. air (B) or in air + 3% CO_2 (A). In the measurements corresponding to curve B the sensitivity of the apparatus was greater than in those corresponding to curve A. *Ordinate*: carbon dioxide uptake in arbitrary units; *abscissa*: time in min. Black and white areas indicate dark and light periods for the plant material respectively.

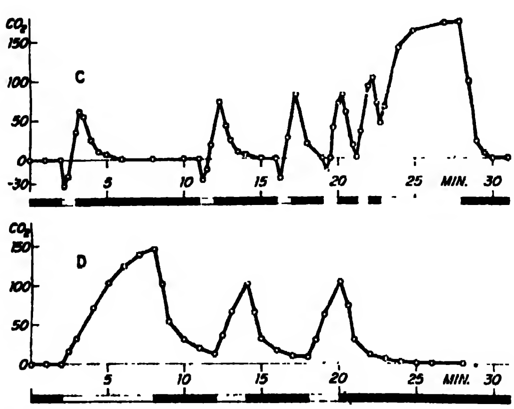

Fig. 2. Time curves for *Ch. pyrenoidosa* cultivated in liquids aerated either with air + 5% CO_2 (C) or with atmospheric air (D). Both curves are measured in air + 3% CO_2. Units as in Fig. 1.

algae were cultivated and measured in high concentrations of carbon dioxide. Light periods of one min duration were followed by dark periods of decreasing lengths (8, 4, 2 min and 75 and 45 sec respectively). As had been shown by Emerson and Lewis[6] and by van der Veen[7], the CO_2-bursts diminish with decreasing dark periods before illumination.

Using algae grown in a solution aerated with atmospheric air instead of algae cultivated in air containing 5% CO_2, and making the measurements in air containing 3% CO_2, we found the time-curve to be divergent (Fig. 2D). Both the CO_2-gush and the induction peak failed to appear when the experiment was carried out under these conditions.

As a result of our experiments we can conclude that in the strain of *Chlorella pyrenoidosa* employed it is possible to demonstrate a burst of carbon dioxide immediately following illumination after a few minutes of darkness. However, this burst occurs only if the following conditions are met: a) The plants must have been cultivated in a liquid medium through which air enriched with carbon dioxide (about 4–5%) is bubbled and b) It is necessary to make the measurements of the photosynthesis in an equally high concentration of carbon dioxide. If either of these conditions is not fulfilled the photosynthesis CO_2-time curve of *Chlorella* shows no sign of a CO_2-gush.

In the strain of *Chlorella* discussed an extraordinarily large amount of carbon dioxide accumulates in the plants under the experimental conditions given above. Measurements of the oxygen exchange have shown that immediately upon illumination the *Chlorella* plants start evolving oxygen in spite of the appearance of a simultaneous burst of CO_2. Thus the plants start photosynthesizing instantly upon illumination; clearly this involves a disturbance in the carbon dioxide equilibrium and results in a discharge of the accumulated carbon dioxide which manifests itself as the carbon dioxide gush.

The *Chlorella* strain used does not seem to show any induction phenomena under natural conditions. Only if the plants are under the influence of extraordinarily high concentrations of carbon dioxide does a very special induction phenomenon, *i.e.* the CO_2-burst, appear.

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KNUD VEJLBY
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[1] K. VEJLBY, *Physiol. Plantarum*, 11 (1958) 158.
[2] K. VEJLBY, *Physiol. Plantarum*, 11 (1958) 866.
[3] K. VEJLBY, *Physiol. Plantarum*, 12 (1959) 559.
[4] K. VEJLBY, *Comm. Intern. Photobiol.*, Bull. No. 3 (1960) 37.
[5] K. VEJLBY, *Nature*, 186 (1960) 652.
[6] R. EMERSON AND C. M. LEWIS, *Am. J. Botany*, 28 (194-) 789.
[7] R. VAN DER VEEN, *Physiol. Plantarum*, 3 (1950) 247.
[8] A. H. BROWN AND C. P. WHITTINGHAM, *Plant Physiol.*, 30 (1955) 231.
[9] R. G. HILLER AND C. P. WHITTINGHAM, *Plant Physiol.*, 34 (1959) 219.
[10] E. D. MCALISTER AND J. MYERS, *Smithsonian Misc. Collections*, 99(6) (1940) 1.
[11] H. GAFFRON, *Research in Photosynthesis*, Natl. Acad. Sci., Natl. Research Council, New York, 1957, p. 430.

The use of ultraviolet irradiation in studies of the mechanism of oxidative phosphorylation*

The effect of U.V. light on isolated mitochondrial systems has been studied as part of a departmental project concerned with the alterations of living systems brought about by such radiations. The results of such studies on isolated mitochondrial systems indicate that U.V. light may be employed as a valuable tool, or a physical "scalpel", with which certain components of the mitochondrial oxidative phosphorylation system may be selectively inactivated, or altered, and studied as to their requirements in the overall system. We have employed two wavelengths in the U.V. region in our studies to date. "Far-U.V." light of approximately 2600 Å and "near-U.V." light of approximately 3600 Å both appear to inactivate certain portions of the oxidative phosphorylation system. For example, far-U.V. irradiation of isolated rat-liver mitochondria results in total inactivation of both the oxidation of succinate and glutamate and phosphorylations accompanying electron transport from these substrates. During such irradiation various nucleotides such as AMP**, ADP, DPN, TPN and P_i are released from the particles. ATP does not appear in the extramitochondrial fluid in quantities in excess of control preparations during irradiation, although the level of intramitochondrial ATP is diminished. This would indicate that ATP, upon being released, is enzymically hydrolyzed to ADP and P_i. Neither oxidation nor phosphorylation, after irradiation with far-U.V. light, is restored by the addition of the released nucleotides or by a variety of other known or suspected cofactors of these systems. Both ATP and crystalline serum albumin afford a high degree of protection during irradiation. Versene, when tested over a wide range of concentrations, does not protect mitochondria during far-U.V. treatment, nor is protection afforded by the presence of disulfide protectors such as cysteine and glutathione.

We have also studied the effect of far-U.V. light on two reactions, occurring in mitochondria, which are thought to be closely related to the process of oxidative phosphorylation. For example, both the ATP-P_i exchange reaction and the DNP stimulated ATPase of mitochondria are inactivated by irradiation. Another ATPase occurring in mitochondria, that which is stimulated by magnesium ions after DOC treatment of the particles, appears to be quite insensitive to the effects of far-U.V. light. From such studies on the ATPases of mitochondria it would appear that far-U.V. light inactivates a component of the overall degradative process concerned with ATP hydrolysis which is not shared by the DNP-stimulatable and the Mg^{++}-stimulatable ATPases. This may be interpreted as indicating that at least two pathways for ATP breakdown exist in mitochondria which share the initial step, or steps, of the reaction sequence, but diverge prior to a step dependent upon the oxidation–reduction state of the electron transport component involved. We would prefer to call the U.V.

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** The following abbreviations are used: AMP, ADP, and ATP, adenosine mono-, di- and triphosphate; DPN and TPN, di- and triphosphopyridine nucleotide; P_i, inorganic orthophosphate; DOC, deoxycholate; DNP, 2,4-dinitrophenol.

insensitive step the "unphysiological" pathway since the ATP-P_i exchange reaction, presumably utilizing the same transphosphorylative components involved in the U.V. sensitive or physiological ATPase sequence, does not appear to be involved in the alternative (U.V. sensitive) degradative reaction. The results of these experiments would also indicate that the U.V.-sensitive component is close to, or at, the electron transport level and might conceivably be involved in energy conservation, *i.e.* the transformation of energy released during the process of electron transport into phosphate bond formation resulting in the synthesis of ATP.

At present, three possible electron transport components are being seriously considered as candidates for energy conservation sites involved in the first phosphorylation coupled to the flow of electrons in the respiratory chain. They are DPN, flavoprotein and a substituted quinone. Although a certain amount of indirect evidence implicating each of these molecules exists, we would like to describe the results of our experiments bearing on the possibility of a quinone involvement. Since far-U.V. light results in a rapid inactivation of the systems studied, we have utilized the longer wavelength of approximately 3600 A which appeared to result in a more gentle, and perhaps more specific, treatment of the mitochondrion. Such near-U.V. treatment results in mitochondria which have lost the ability to oxidize succinate and glutamate and to carry out oxidative phosphorylation. Oxidative phosphorylation with succinate as substrate is restored to near control values by the addition of cytochrome *c*, and oxidative phosphorylation with glutamate as substrate is dependent upon the addition of cytochrome *c* and vitamin K_1. We have tested a number of quinones in regard to their ability to restore oxidation and phosphorylation, and of those quinones only vitamin K_1 acetate and a lipid extract of *Mycobacterium phlei* are capable of effecting slight restoration. The inhibitors DNP and Dicumarol uncouple all phosphorylations in the restored systems, and amytal, a known inhibitor of electron transport from DPN, blocks electron transport in the vitamin K_1-restored system. These data indicate that near-U.V. light treatment of mitochondria alters a factor functioning in oxidative phosphorylation between DPN and cytochrome *b* which may be replaced by vitamin K_1, and thus strongly implicates a quinone, with structural similarities to vitamin K_1, as participating in mitochondrial energy conservation.

We are at present attempting to study reactions related to oxidative phosphorylation in more purified systems and have been successful in obtaining a soluble ATPase system from sonic extracts of mitochondria. Since the soluble ATPase system is stimulated by DNP we are hopeful that we are dealing with a physiological pathway, the components of which we hope to be able to analyse. We thus consider U.V. light as an extremely valuable tool in the study of the mechanisms involved in the conservation of energy during the process of mitochondrial oxidative phosphorylation.

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Session 4

THE REACTION OF THE SKIN TO RADIATION

Chairman: W. BURCKHARDT, Zürich (Switzerland)

Secretary: ERIK KNUDSEN, Copenhagen (Denmark)

Die optischen Eigenschaften der menschlichen Haut im IR-Bereich

Die Kenntnis der Emissionseigenschaften der lebenden menschlichen Haut im infraroten Spektralbereich gestattet die Berechnung der unter bestimmten Temperaturverhältnissen durch Wärmestrahlung an die Umgebung abgegebenen Energie. Umgekehrt lässt sich aus Strahlungsmessungen die Hautoberflächentemperatur berechnen, wenn die Abweichungen der Haut vom schwarzen Körper bekannt sind. Reflexion und Absorption von IR-Strahlung geben Auskunft über das Schicksal der von der Umgebung auf die Haut fallenden Wärmestrahlung.

Methodik

Zur Durchführung von Emissions-, Reflexions- und Durchlässigkeitsmessungen wurde ein universeller IR-Messplatz zusammengestellt. Als Strahlungsquellen dienten für die Reflexions- und Durchlässigkeitsmessungen ein Silitstab (Globarstrahler), der elektrisch auf *ca.* 1200° geheizt wurde, für Emissionsmessungen die Volarseite des Unterarmes sowie ein schwarzer Körper, der durch thermostatisiertes Wasser auf Hauttemperatur eingeregelt werden konnte. Die Strahlung wurde mit einer Sektorenscheibe mit 12.5 Hz moduliert, damit das Thermoelement eine Wechselspannung abgibt. Eine Kammblende erlaubte die messbare Schwächung der einfallenden Strahlung. Die spektrale Zerlegung erfolgte in einem Spiegelmonochromator, der bis λ 5 μ mit einem LiF Prisma und für grössere Wellenlängen mit einem NaCl-Prisma ausgerüstet war. Als Nachweisinstrument diente ein Vakuum-Thermoelement niedriger Zeitkonstante mit KBr-Linse; die Empfindlichkeit beträgt 12 V/W. In einem Vorverstärker erfolgte die Anpassung des Thermoelementes an den Röhrenverstärker und die Vorverstärkung. Die Nachverstärkung geschah in einem frequenzselektiven Hauptverstärker und in einem phasenempfindlichen Gleichrichter die Demodulation. Ein Tintenschreiber an dessen Ausgang ermöglichte die Aufzeichnung der Spektren. Zwischen Monochromatoraustrittsspalt und Thermoelement befand sich bei den Reflexionsmessungen eine integrierende Halbkugel, die die von der Hautmessstelle diffus reflektierte Strahlung nach einmaliger Reflexion an der verspiegelten Innenwand auf das Thermoelement konzentriert. Bei den Durchlässigkeitsmessungen befand sich direkt vor dem Thermoelement die Hautprobe.

Zu den Emissionsmessungen

In der Literatur sind an experimentellen Arbeiten über die Spektralverteilung der von der Haut an die Umgebung abgegebenen Energie nur die von Hardy und Muschenheim[1] aus dem Jahre 1934 bekannt. Die Emissionswerte der Haut und die eines schwarzen Körpers überkreuzen sich einige Male, die Abweichungen betragen bis 20%, obwohl die Autoren im Text schreiben, beide Kurven unterschieden sich nur unwesentlich.

Die Ergebnisse der eigenen Messungen sind in Abb. 1 zusammengefasst. Hier ist die Spektralverteilung der von der Haut bzw. einem schwarzen Körper von *ca.* 32° an die Umgebung von *ca.* 20° durch Strahlung abgegebenen Energie dargestellt. Kurve 1

Literatur S. 491

zeigt die Mittelwerte aus 8 Messreihen an zwei Versuchspersonen, Kurve 2 stellt die Mittelwerte aus ebenfalls 8 Messreihen am schwarzen Körper dar und Kurve 3 ist die aus theroretischen Überlegungen erwartete. Letztere errechnet sich aus der Differenz der Planck'schen Strahlungskurven für Haut- und Umgebungstemperatur für jede Wellenlänge. Die Abweichungen der gemessenen Werte von den theoretischen ab

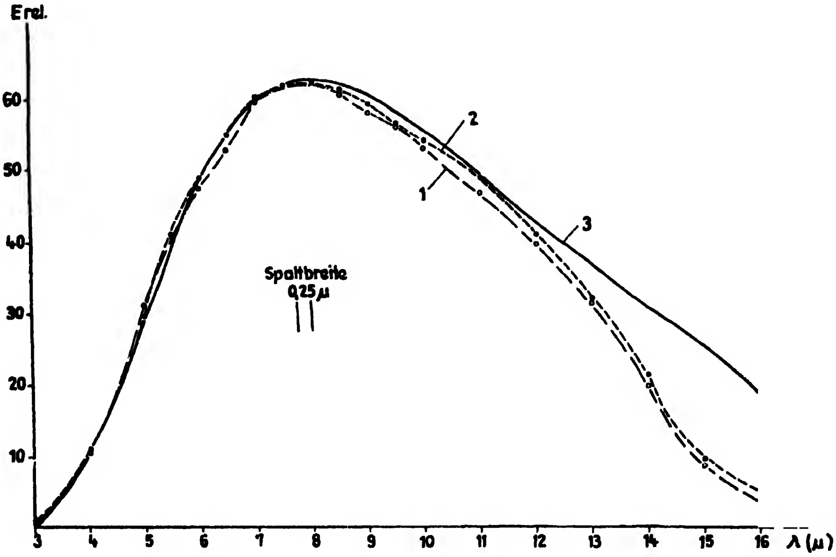

Abb. 1. Spektralverteilung des Energieverlustes durch Wärmestrahlung von Haut und schwarzem Körper. 1. Hautstrahlung, $T_H = 32°$, $T_U = 20°$, 2. Schwarzer Körper, gemessen, $T_{SK} = 32°$, $T_U = 20°$, 3. Schwarzer Körper, berechnet, $T_{SK} = 32°$, $T_U = 20°$.

$12\,\mu$ sind auf Absorption des NaCl-Prismas zurückzuführen. Geringfügige Abweichungen der Haut vom schwarzen Körper bei $6\,\mu$ und von 9 bis $11\,\mu$ könnten durch Wasserabsorption bedingt sein. Danach ist die Haut als grauer Temperaturstrahler anzusehen. Genaue Aussagen über den Unterschied im Emissionsvermögen von Haut und schwarzem Körper lassen sich wegen der unbefriedigenden Genauigkeit thermoelektrischer Oberflächentemperaturmessungen nicht machen.

Zu den Reflexionsmessungen

In der Abb. 2 sind die Mittelwerte des ermittelten Reflexionsvermögens aus 10 Messreihen an zwei Versuchspersonen zusammen mit den bisher in der Literatur vorliegenden Messungen dargestellt. Die Lage der Selektivitäten ist einheitlich; sie sind durch Wasserabsorption bedingt. Die Absolutwerte schwanken im kurzwelligen Bereich stark, da unterschiedliche Hautdicken benutzt wurden. Da das Reflexionsspektrum die Wasserabsorptionsbanden enthält, ist es naheliegend, im Wassergehalt der obersten Hautschichten den für die optischen Eigenschaften massgeblichen Faktor zu suchen. Das mittlere Reflexionsvermögen über den vermessenen Spektralbereich ergab sich unter Berücksichtigung der Spektralverteilung der Emission zu $6.12 \pm 0.6\%$. Weiterhin wurde das Reflexionsvermögen ohne spektrale Zerlegung über den gesamten an der Abstrahlung beteiligten Bereich (von $3\,\mu$ = untere Emissions-

wellenlänge bis 30 μ = Grenze der Durchlässigkeit der KBr-Linse des Thermoelementes) gemessen. Als Strahlungsquelle diente hierbei der schwarze Körper von 32°, damit die Spektralverteilung der Messstrahlung derjenigen entspricht, die die Haut emittiert. Es ergab sich ein Mittelwert aus 40 Messungen von 6.1 + 0.3%.

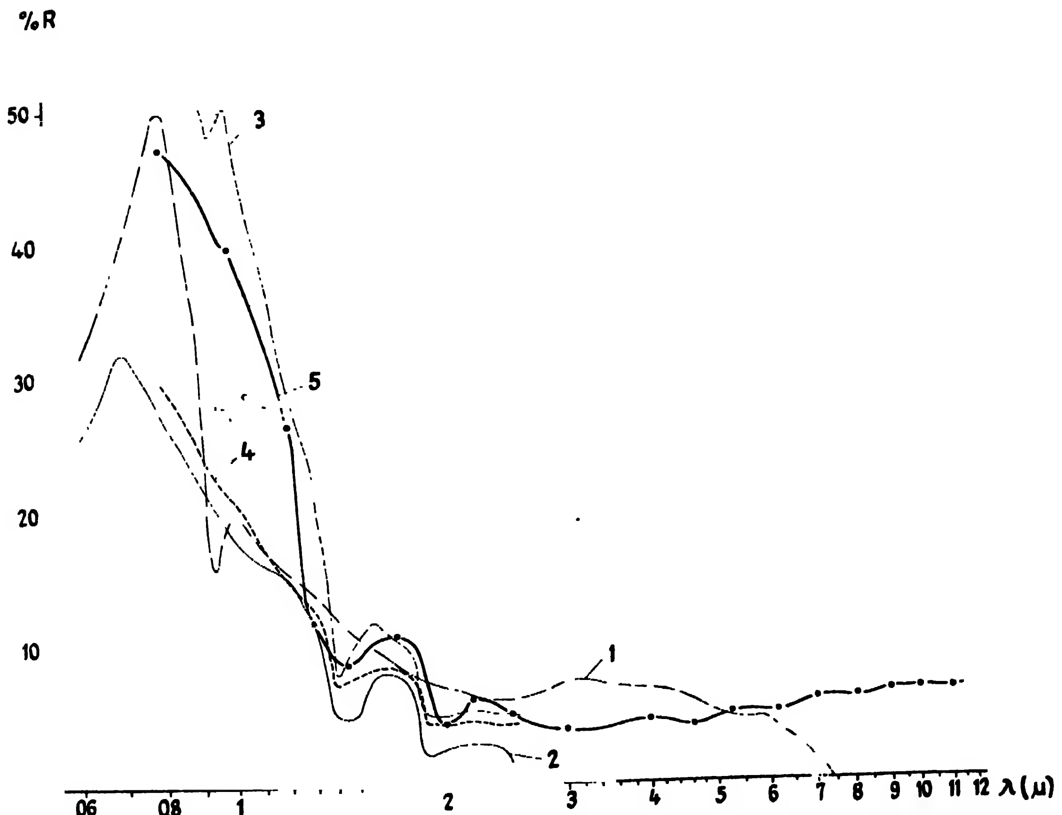

Abb. 2. Spektrales Reflexionsvermögen der menschlichen Haut. (1) Hardy und Muschenheim[1]; (2) Clark, Vinegar und Hardy[2]; (3) Jacquez u.a.[3]; (4) Hardy, Hammel und Murgatroyd[4]; (5) Eckoldt, 1960.

Dieser Wert ist abhängig vom Feuchtigkeitsgehalt der Haut. Befeuchtet man die Hautmessstelle leicht, wie es einer mittleren sensiblen Perspiration entspricht, so sinkt das Reflexionsvermögen auf Werte um 4% ab, um im Verlauf der Trocknung wieder auf *ca.* 6% anzusteigen.

Der Einfluss der Durchblutung auf das Reflexionvermögen wurde geprüft, indem einmal Wärme- und UV-Erytheme gesetzt wurden und zum anderen die Durchblutung des Armes mit einer Staumanschette am Oberarm gedrosselt wurde. Hierbei ergaben sich keine Unterschiede im Reflexionsvermögen.

Zu den Durchlässigkeitsmessungen

Die Durchlässigkeit lebender, durchbluteter menschlicher Haut (Skrotum, *ca.* 2 mm dick) konnte bis $\lambda \leqslant 1.8\,\mu$ gemessen werden. Weiterhin wurden *ca.* 0.2 mm dicke

Literatur S. 491

Hautschichten vermessen, die durch Mikrotomschnitte von frisch abgenommenen Operationsteilen (Mamma) gewonnen wurden. Die Messungen wurden im Zeitraum von 1 bis 4 Stunden nach der Resektion durchgeführt, Austrocknung wurde nach Möglichkeit vermieden. Die erhaltenen spektralen Durchlässigkeitswerte wurden nach Abzug des reflektierten Anteils auf natürliche Extinktionsmoduln (Extinktion pro Einheit der Schichtdicke) umgerechnet. In der Abb. 3 sind diese Werte zusammen

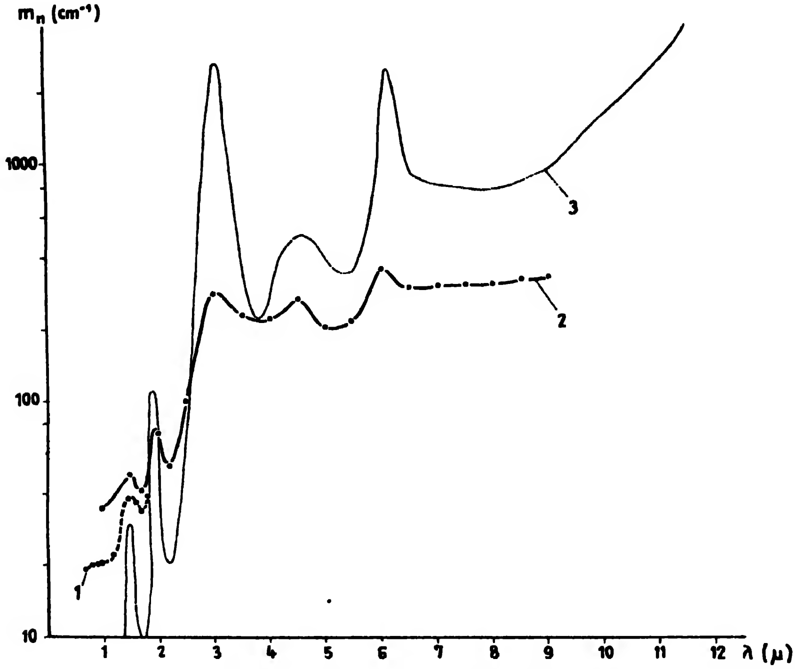

Abb. 3. Extinktionsmoduln der menschlichen Haut und des Wassers. (1) Haut, Skrotum; (2) Haut, isolierte Schicht; (3) Wasser.

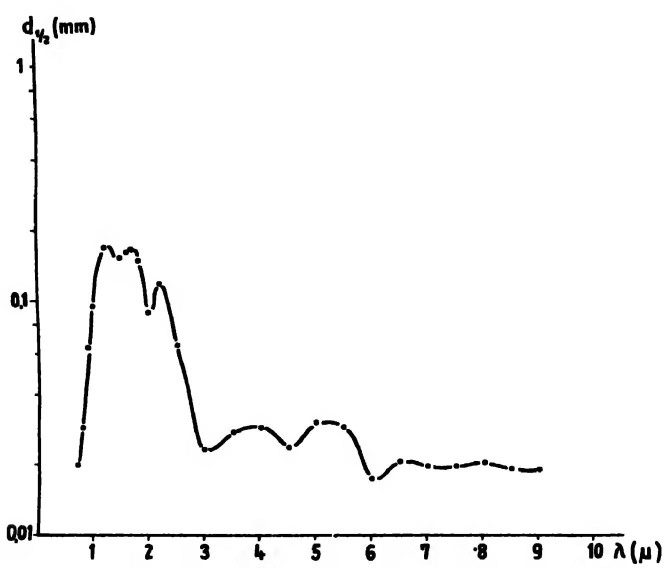

Abb. 4. Halbwertsdicken der Haut gegen IR-Strahlung.

mit den Wasserextinktionskoeffizienten dargestellt. In der Abb. 4 sind dann die Schichtdicken aufgezeichnet, in denen die Hälfte der auffallenden Intensität absorbiert wird (Halbwertsdicke). Hierbei wurde der Energieverlust durch Reflexion an der Oberfläche mit berücksichtigt.

Aus den vorliegenden Messungen werden folgende Schlussfolgerungen gezogen:

1. Die Haut ist im Bereich von 3 bis 16 μ als grauer Temperaturstrahler anzusehen.
2. Das Reflexionsvermögen und damit auch das relative Emissionsvermögen (emissivity) ist abhängig vom Feuchtigkeitsgehalt der obersten Hautschichten. Eine Abhängigkeit von der Durchblutung konnte nicht nachgewiesen werden.
3. Die Temperatur tiefer gelegener Hautschichten kann die Oberflächentemperatur nicht beeinflussen.
4. Nur einfallende Strahlung aus dem Wellenlängenbereich von 1 bis 1.8 μ kann die Epidermis durchdringen.

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[1] J. D. HARDY UND K. MUSCHENHEIM, *J. Clin. Invest.*, 13 (1934) 817.
[2] C. CLARK, R. VINEGAR UND J. D. HARDY, *J. Opt. Soc. Am*, 43 (1953) 993.
[3] J. A. JACQUEZ, J. HUSS, W. MCKEEHAN, J. M. DIMITROFF UND H. F. KUPPENHEIM, *J. Appl. Phys.* [illegible] (1955) 297.
[4] J. D. HARDY, H. T. HAMMEL UND D. MURGATROYD, *J. Appl. Physiol.*, 9 (1956) 257.

Neuere Ergebnisse zur Erythem- und Pigmentwirkung optischer Strahlung

Der Abhängigkeit des UV-Erythems von der Wellenlänge wurden schon zahlreiche Untersuchungen gewidmet. Auf dieser Grundlage wurde von der Internationalen Beleuchtungskomission (IBK) die Wellenlängenabhängigkeit für ein mittleres Erythem empfohlen. Diese Abhängigkeit, die meist linear dargestellt wird, vernachlässigt aber in dieser Art der Darstellung die Erythemwirkung der Strahlung im UV-A bei Wellenlängen über 315 mμ. Auch die Erythemwirkung in diesem längerwelligen Gebiet ist schon des öfteren untersucht worden. Die Ergebnisse waren aber sehr unterschiedlich. Messungen der Erythemwirksamkeit in diesem Bereich sind nämlich sehr schwierig durchzuführen. Die Wirksamkeit dieser Strahlung ist um Grössenordnungen kleiner als die der kurzwelligen Strahlung, und Streulicht dieser Strahlung muss also in höchstem Masse ausgeschlossen werden. Die Kenntnis der Erythemwirksamkeit in diesem Gebiet ist aber dennoch von grosser Bedeutung, da der spektrale Anteil des UV-A bei der Strahlung der Sonne und auch bei den therapeutischen Hg- und Xe-Strahlern viel grösser ist als die der kürzerwelligen Strahlung.

Ferner muss, wenn die UV-Erythemempfindlichkeit sehr niedrig ist, zur Erzielung eines Erythems die Bestrahlungsstärke stark erhöht werden. Dadurch ist es gegebenenfalls möglich, dass durch die absorbierte Energie in der Haut, die Temperatur sich soweit erhöht, dass ein Wärmeerythem entsteht. Dieses hat die Aufgabe, durch die höhere Blutzirkulation die absorbierte Energie abzuführen und möglichst zu verhin-

dem, dass Verbrennungen entstehen. Dieses Erythem ist unspezifisch, d.h. an keinen Wellenlängenbereich, also auch nicht an die sogenannte Wärmestrahlung, gebunden.

Dieses Erythem, das wir auch unspezifisches Erythem nennen können, unterschei-

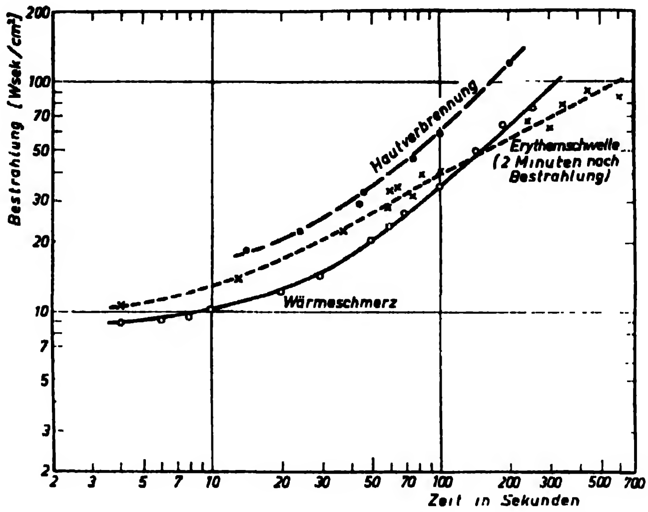

Abb. 1. Die Bestrahlung in Abhängigkeit von der Bestrahlungszeit für Schmerzschwelle, Erythemschwelle und Verbrennungsschwelle.

det sich vom UV-Erythem durch seine kleinere Latenzzeit. Es erscheint nach wenigen Minuten. Dies bedingt, dass schon bei Bestrahlungszeiten, die nur grösser als 10 Sekunden sind, die Wirkung sowohl von der Bestrahlung als auch von ihrer zeitlichen Verteilung, also von der Bestrahlungsstärke abhängig ist. Dies ist in der ersten Abbildung dargestellt.

Man erkennt, dass der Verlauf dieser Abhängigkeit für die Erythemschwelle eine andere ist, als für die Schmerzschwelle und die Verbrennungsschwelle.

Die Abklingzeit des unspezifischen Erythems ist im allgemeinen kleiner als die des spezifischen UV-Erythems. Deshalb gelingt es diese Erytheme in einem gewissen Bereich voneinander zu unterscheiden. Dieser Bereich wird jedoch dadurch eingegrenzt, dass die Abklingzeit des unspezifischen Erythems sowohl vom Erythemgrad als auch von der zeitlichen Entstehung des Erythems abhängt Starke Erytheme halten eine längere Zeit an, und eine Trennung des spezifischen Erythems vom unspezifischen ist nicht mehr möglich. Die Grenze der Trennbarkeit liegt etwas unterhalb der Verbrennungsschwelle. Bei kurzen Bestrahlungszeiten ist der Unterschied zwischen Erythem und Verbrennung nicht sehr gross. Die Gradation der Effekte ist steil. Der Unterschied nimmt erst bei Bestrahlungszeiten über 1 Minute zu.

Zur Abgrenzung des UV-Erythems bei längeren Wellenlängen ist in der nächsten Abbildung der Verlauf des UV-Erythems zusammen mit dem unspezifischen Erythem und der Verbrennungsschwelle aufgezeichnet. Die unspezifische Erythemschwelle ist dabei für drei Einwirkungszeiten, und zwar 1 Sekunde, 1 Minute und 20 Minuten gemäss der Abhängigkeit der ersten Abbildung, eingezeichnet.

Man erkennt, dass bei Bestrahlungszeiten bis zu 1 Minute und bei Wellenlängen über 330 mμ die beiden Erytheme nicht mehr unterschieden werden können. Erst bei Bestrahlungszeiten von etwa 20 Minuten und darüber scheint eine Trennung möglich.

Bei diesen längeren Bestrahlungszeiten nimmt jedoch auch die Abklingzeit für das unspezifische Erythem zu. Die Abklingzeit für ein unspezifisches Erythem liegt nämlich ungefähr in derselben Grösse wie die Entstehungszeit. Man gerät deshalb bei längeren Bestrahlungszeiten in die Latenzzeit des UV-Erythems. Es folgt deshalb

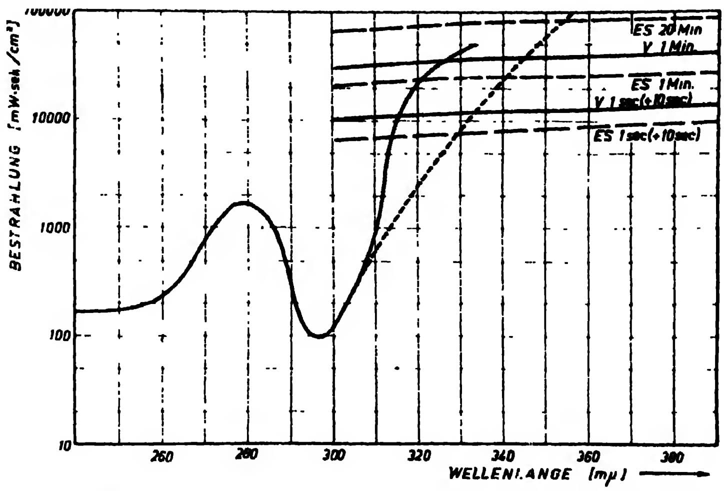

Abb. 2 Die Bestrahlung für ein mittleres UV-Erythem (nach IBK Mittleres Erythem bei 297 mμ gleich 100 mW·sec/cm² gesetzt) und für die Erythem- und Verbrennungsschwelle beim unspezifischen Erythem für verschiedene Bestrahlungszeiten.

hieraus, dass bei Strahlung über etwa 340 mμ ein spezifisches Erythem als solches nicht mehr sicher definiert werden kann. Ferner ist der Übergang zwischen UV und unspezifischem Erythem abhängig sowohl von der Bestrahlung als auch von der Bestrahlungsstärke. Durch eine einzige Kurve kann dieser Übergang nicht dargestellt werden.

Diese Ergebnisse konnten wir ableiten aus Messungen des unspezifischen Erythems im sichtbaren Bereich und Übertragung der Ergebnisse auf den UV-Bereich. Das unspezifische Erythem hängt nämlich, wie wir im Spektralbereich von 0.4-1.4 μ feststellen konnten, nur von der Absorption der Haut ab. In der obigen Abbildung ergibt sich die Wellenlängenabhängigkeit des unspezifischen Erythems aus der spektralen Reflexion der Haut.

Im UV-A liegt aber auch das Gebiet der direkt pigmentierenden Wirkung. Über die Wellenlängenabhängigkeit und die Empfindlichkeit dieser Wirkung sind besonders von Henschke und Schulze und I. Hausser als ersten genaue Untersuchungen durchgeführt worden. Wir fanden, dass die Bestrahlungsdosis für ein direktes Pigment bei der Wellenlänge 350 mμ *ca.* 300 bis 1000 mal grösser ist als die Bestrahlungsdosis für ein Erythem durch die Wellenlänge 297 mμ. Man kommt deshalb auch leicht in den Bereich des Auftretens eines unspezifischen Erythems. Bei allen Untersuchungen zu diesen Fragen muss man dies nach der oben gezeigten Darstellung berücksichtigen.

Unsere Untersuchungen wurden mit einem besonders leistungsfähigen Monochromator durchgeführt. Durch zusätzliche Sperrfilter wurde Sorge dafür getragen, dass kürzerwelliges Streulicht nicht zur Wirkung kam. Die spektrale Verteilung der austretenden Strahlung wurde, um dies zu kontrollieren, auch stets ausgemessen.

Damit konnten wir die bisher bekannte Wellenlängenabhängigkeit im wesentlichen

bestätigen. Wir fanden, dass bei einer Wellenlänge von 350 mμ ein Maximum der pigmentierenden Wirkung liegt. Als Pigmentschwellendosis fanden wir für Personen, die (nach Schulze) sowohl mit Pigment als mit Erythem reagieren, eine Bestrahlung von 6 W·sec/cm². Für Personen, die im wesentlichen nur pigmentempfindlich sind, lag die Schwellendosis $\lambda = 350$ mμ bei 2 W·sec/cm² $\pm 15\%$. Diese Personengruppe ist also 3 mal empfindlicher für eine Pigmentierung als Personen, die auch erythem-empfindlich sind.

In diesem Bereich, also bei einer maximalen Wirksamkeit der Strahlung, konnten wir dank der hohen zur Verfügung stehenden monochromatischen Bestrahlungsstärke schon Pigmente bei Bestrahlungszeiten von 3 Min. erzielen. Diese bei kurzen Bestrahlungszeiten erzielten Pigmente klingen jedoch, falls nicht weiter bestrahlt wird, schnell wieder ab. Bereits nach 20 Min. sind diese durch kurze Bestrahlungszeiten erzeugten Pigmente völlig verschwunden. Das bedeutet also, dass zu einer Manifestierung des Pigmentes es einer genügend langen zeitlichen Einwirkung der Bestrahlung von mindestens 10 Min. bedarf. Dies steht im Einklang damit, dass sich die dabei verlaufenden Oxidationsprozesse in mehreren Stufen vollziehen. Ferner ist dies in Übereinstimmung mit der alten Beobachtung, dass bei der Verhinderung der Blutzirkulation durch Kompression keine Pigmentierung auftritt. Dasselbe ist also auch der Fall, wenn die Zeit während der Bestrahlung nicht ausreicht, um genügend Sauerstoff für den Ablauf sämtlicher Prozesse heranzuschaffen. Betrachtet man die Abhängigkeit der Bestrahlungsdosis für ein Pigment von der Bestrahlungszeit, so ergibt sich hieraus ein Unterschied, je nachdem nach welcher Zeit die Pigmentbeurteilung vorgenommen wird, die in der nächsten Abbildung dargestellt ist.

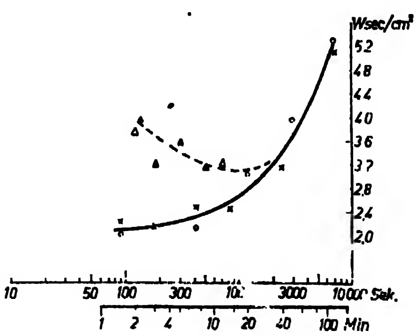

Abb. 3. Abhängigkeit der Bestrahlung von der Bestrahlungszeit für die Pigmentschwelle. Ausgezogene Kurve Ablesezeit 3 Min. nach Bestrahlungsende. Gestrichelte Kurve 60 Min. nach Bestrahlungsende.

Es ergibt sich, dass zu einem gleichstarken Pigment, das 1 Stunde nach der Bestrahlung beobachtet wird, bei kurzer Bestrahlungszeit eine grössere Bestrahlung erforderlich ist als bei längerer Bestrahlungszeit. Der Zeitfaktor ist kleiner als eins. Die Ursache hierfür ist, dass die Zeit, die notwendig ist, um die Reaktionspartner zusammenzuführen, in der Grössenordnung der Bestrahlungszeit liegt.

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HORST BÜCKER

Untersuchungen über das Reflexions- und Emissionsvermögen sowie die Durchlässigkeit der lebenden menschlichen Haut im Spektralbereich $\lambda \approx$ 3–15 μm*

Im vorangegangenen Vortrag von Herrn Eckoldt wurde herausgestellt, dass bei Zimmertemperatur das Maximum der Temperaturstrahlung eines experimentellen schwarzen Körpers von etwa 32°C, wie auch das der lebenden menschlichen Haut nicht, wie dem Planck'schen Gesetz gemäss zu erwarten, bei $\lambda \approx 9.4$ μm, sondern bei $\lambda \approx 8.0$ μm liege. Eigene Versuche brachten zunächst ähnliche Ergebnisse. Es wurden ebenfalls der von Falta[1] näher beschriebene Spiegelmonochromator des VEB Carl Zeiss Jena mit einem 67°-UR-Steinsalzprisma sowie das wohl grundsätzlich im Jahre 1931 von Nicolai[2,3] eingeführte und später von Lehrer[4,5] in die Ultrarottechnik übernommene Wechsellichtverfahren benutzt. Als Empfänger dienten Bolometer nach Czerny, Kofink und Lippert[6]** mit Wismutaufdampfungen in der Woltersdorff' schen Schichtdicke[7] als Brückenglieder. Zur Verstärkung diente ein Resonanzverstärker** mit fünf, davon vier auf 12.5 Hz abgestimmten Niederfrequenzkreisen mit Spannungskonstanthaltung. Eine lineare Nachverstärkung gestattete den Betrieb geeigneter Schreiber zur Aufzeichnung der Verteilungskurven im Spektralbereich zwischen etwa λ = 3-15 μm.

Czerny[8] wies bereits im Jahre 1944 auf Fehlerquellen hinsichtlich des scheinbaren Auswanderns des Maximums hin, wenn bei Zimmertemperatur nur wenig höher temperierte Strahler mit Prismenmonochromatoren gemessen werden. Ein ganz erheblicher Fehler ergibt sich aber unter solchen Bedingungen, wenn bei Einsatz des Wechsellichtverfahrens im Ultrarotbereich nicht beachtet wird, dass die registrierten Werte die Differenz zwischen zwei Strahlern ähnlicher Temperatur, nämlich der des gemessenen Objektes und der zur Modulation benutzten Unterbrecherscheibe wiedergeben. Berechnungen nach dem reduzierten Planck'schen Gesetz ergeben bei Zugrundelegung einer Raumtemperatur von 293°K, die für die Modulationsscheibe ebenfalls gelten soll, sowie einer Haut- oder Eichstrahlertemperatur von 308°K eine Differenzkurve, die ihr Maximum bei λ = 8.4 μm aufweist. Setzt man dagegen für die Modulationsscheibe eine Temperatur von nur 223°K ein, so liegt das Maximum der Differenzkurve, wie gefordert, bei $\lambda \approx 9.4$ μm. Die Planck'sche Verteilungskurve für die höhere Temperatur und die Differenzkurve weichen dann nur noch im langwelligen Schenkel voneinander ab. Völlige Übereinstimmung kann naturgemäss nur dann erwartet werden, wenn es möglich wäre, die Unterbrecherscheibe so tief zu temperieren, dass sie im Bereich der Hautemission nicht mehr selbst strahlt.

Da alle Objekte vor der modulierenden Scheibe Strahlung zum Messprozess beisteuern, muss durch geeignete, durch Tiefkühlung nicht mehr störende Blenden, der Strahlengang eingeengt werden.

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* Eine ausführlichere Darstellung des vorgetragenen Fragenkomplexes erschien in *Pflügers Arch. ges. Physiol.*, 272 (1961) 360.

** Hersteller Physikalisch-technische Werkstätten, Prof. Dr.-Ing. W. Heimann G.m.b.H., Wiesbaden-Dotzheim.

Literatur S. 501

Abb. 1 veranschaulicht im Schema die aus den dargelegten Gesichtspunkten resultierende Messanlage, die drei Neuerungen bietet:

(*a*) Eine Kühlblende wird durch Alkohol von ca. 230°K, der von einem Trockeneis gekühlten Umlaufthermostaten nach Höppler stammt, durchflossen und eliminiert durch seine Oberflächentemperatur von rund 240°K, den grössten Teil der störenden Umweltstrahlung aus dem Messstrahlengang.

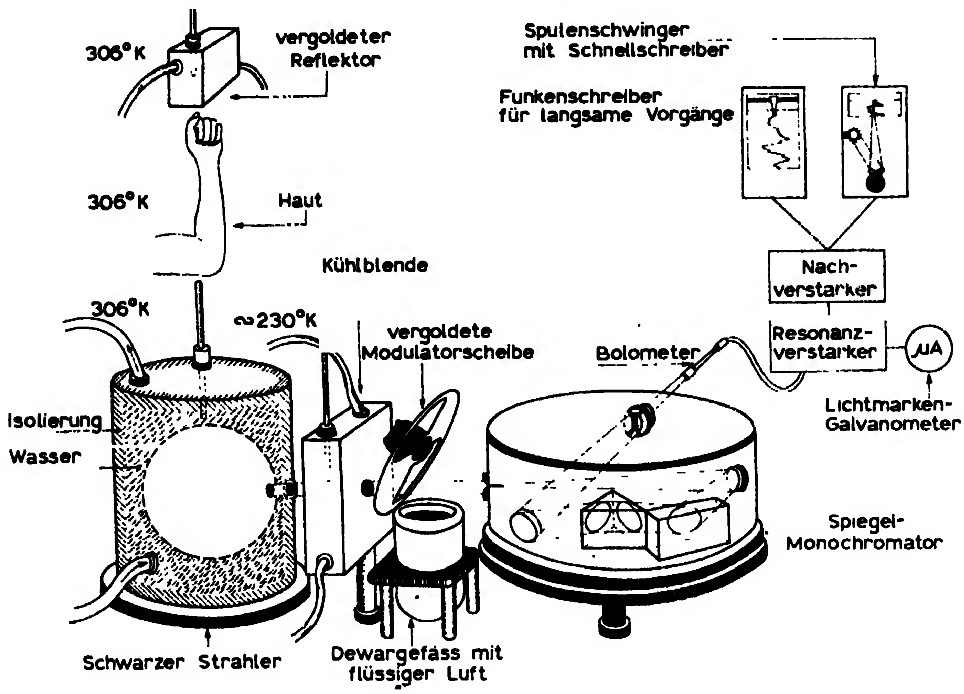

Abb. 1. Schema des Messplatzes für Emissionsmessungen. Vor die Kühlblende werden abwechselnd der schwarze Strahler, die Haut und ein vergoldeter Reflektor gleicher Temperatur gebracht.

(*b*) Eine um 45° geneigte, hochglanzvergoldete Modulationsscheibe gibt im vollen Sektor nicht ihre eigene Temperaturstrahlung in den Monochromator, sondern spiegelt die Oberfläche flüssiger Luft aus einem Dewar-Gefäss als Vergleichsstrahler in den Messweg.

(*c*) Neben der zu messenden Haut und einem idealisierten schwarzen Eichstrahler wird als dritte Grösse ein ebenfalls hochglanzvergoldeter Quader gleicher Temperatur ausgemessen, der auf Grund seines sehr guten Reflexionsvermögens nur die restliche, trotz der beschriebenen Massnahmen noch nicht vermeidbare Störstrahlung anzeigt.

Abb. 2 zeigt einen Ausschnitt des Aufbaues mit der stark vereisten Kühlblende, der schräg gestellten Modulationsscheibe sowie dem Dewar-Gefäss mit flüssiger Luft.

Abb. 3 beweist den fast übereinstimmenden Verlauf der Strahlungskurven des schwarzen Vergleichsstrahlers und der Haut. Die Verteilungskurve des gleich temperierten, vergoldeten Quaders hat dagegen einen wesentlich flacheren Verlauf und zeigt damit, dass sich Abweichungen im Emissionsvermögen sehr deutlich bemerkbar machen. Die Haut muss demnach ein nahezu schwarzer Strahler sein. Die geringen

Abb. 2. Hochglanzvergoldete, um 45° geneigte Modulationsscheibe mit Synchronmotor im Strahlengang zwischen Kühlblende und Monochromator sowie Dewar-Gefäss mit flüssiger Luft.

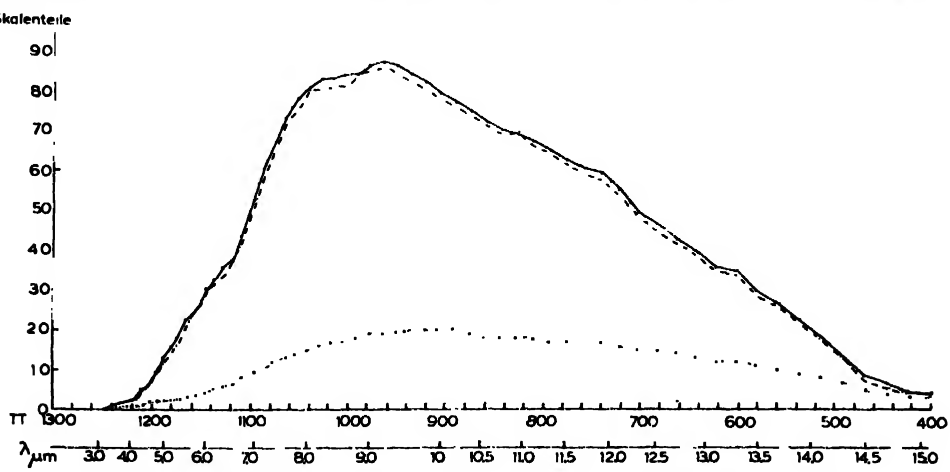

Abb. 3. Ergebnis der Messungen des Emissionsvermögens der lebenden menschlichen Haut im Vergleich zu einem schwarzen Vergleichsstrahler und einen hochglanzvergoldeten Quader. Werte aus neun vollständigen Messreihen. Temp. des schwarzen Körpers 33.7°C – — ; Durchschnittstemp. der Haut 33.3°C -· · · ·; Temp. des goldenen Körpers 33.7°C ·········.

Literatur S. 501

Abweichungen zwischen der Haut- und der Eichstrahlerkurve konnten bei punktförmigen Einzelmessungen ausgeglichen werden. Bei länger dauernden Messungen sinkt dagegen die Hauttemperatur durch die Einwirkung der Kühlblenden und es kommt der geringe Unterschied im Kurvenverlauf zustande. Die Kontrollen der Oberflächentemperatur erfolgten mit geeichten Thermoelementen kleinster Lötstellen, deren Zuführungen 0.05–0.1 mm stark sind und ohne messbaren Auflagedruck jederseits der Hautlötstelle noch 3 cm innerhalb der Isotherme verlaufen.

Eichungen des Prismenmonochromators zum Ausschluss der als bekannt vorausgesetzten typischen Fehlerquellen wurden den Tabellen von Mecke, v. Angerer/Mannkopff und Seidel[9] sowie Angaben von Kortüm[10] und Brügel[11] folgend mittels der Linien- bzw. Bandenspektren eines Quecksilberhochdruckbrenners, von Chloroform, Benzol, Methanol, Äthanol, Trichloräthylen und Aqua dest. sowie den athmosphärischen Wasser- und Kohlendioxydbanden durchgeführt und ergaben mit 0.3 μm bei $\lambda \approx 4.5$ μm maximale Tabellenabweichungen. Sie wurden in der Abb. 3 bereits berücksichtigt. Das Emissionsmaximum der Haut und des schwarzen Eichstrahlers liegen demnach der Theorie entsprechend bei $\lambda \approx 9.4$ μm. Das im Bolometer entstehende Potential beträgt, Eichmessungen mit einem RC-Generator zufolge, in diesem Bereich bei einer spektralen Bandbreite von 0.5 μm etwa $5 \cdot 10^{-8}$ V.

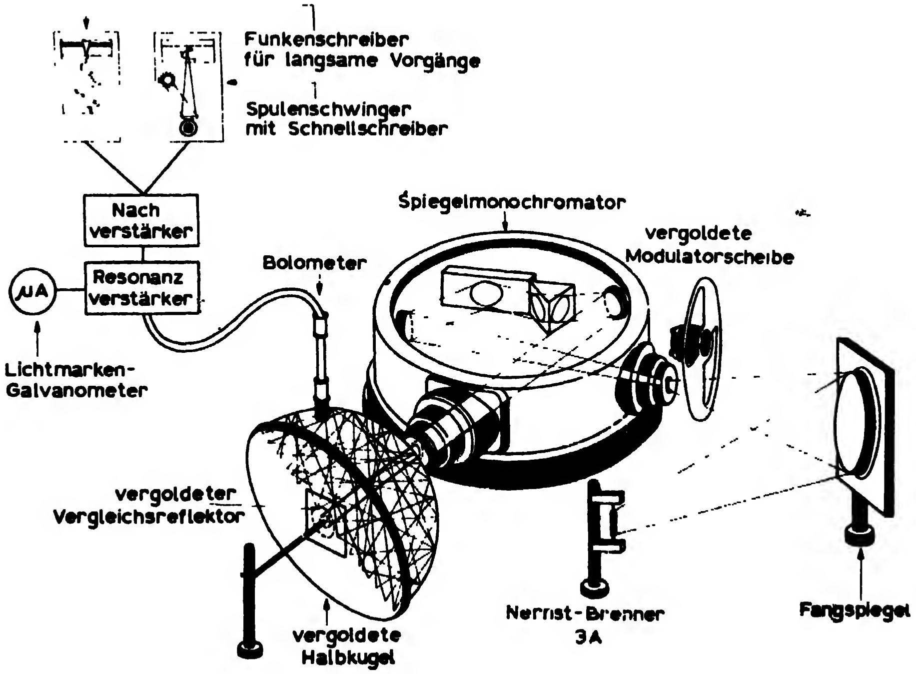

Abb. 4. Schema des Messplatzes für Reflexionsmessungen.

Reflexionsmessungen der lebenden menschlichen Haut wurden mit einer in Abb. 4 schematisch dargestellten Anlage durchgeführt. Als Strahlungsquelle diente ein Nernstbrenner* einer Leistung von 240 W bei 3 A, der mit Hilfe eines Fangspiegels scharf auf dem Eintrittsspalt des Monochromators abgebildet wurde. Das zerlegte

* Hersteller Dr. L. Glaser, Berlin-Altglienicke, Germanenstr.

Licht fällt durch einen Schlitz in eine innen hochglanzvergoldete Halbkugel, verlässt diese entweder auf der gegenüberliegenden Planseite durch eine kreisrunde Öffnung, was einer Totalabsorption entspricht, oder wird im Wechsel von einem ebenfalls vergoldeten Vergleichsreflektor bzw. einer Hautpartie in die Halbkugel hinein reflektiert. In das Rund der Halbkugel ist das Eintrittsfenster des Bolometers harmonisch eingefügt. Durch mehrfache Reflexionen entsteht innerhalb der Halbkugel, das Prinzip des schwarzen Strahlers umkehrend, ein Strahlungsgleichgewicht. Das

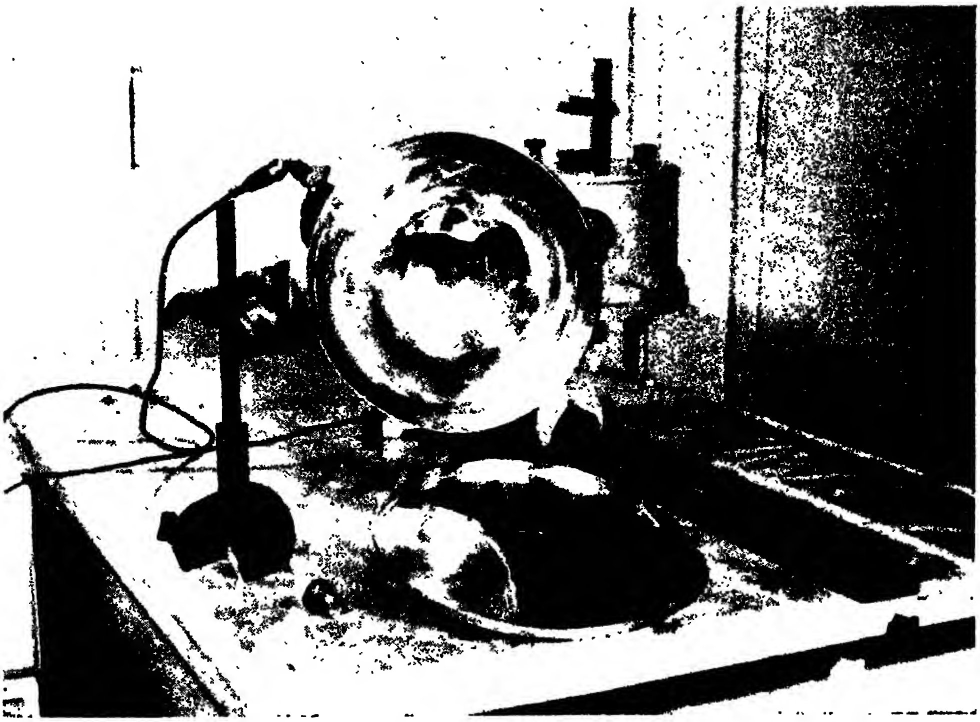

Abb. 5. Innenansicht der im Innern hochglanzvergoldeten Halbkugel nach deren Zerlegung. Man erkennt im Deckel die Austrittsöffnung der Halbkugel, vor die abwechselnd der Vergleichsreflektor und die zu messende Hautpartie gebracht werden. Im Innern der Kugel drei Öffnungen, deren mittlere von der Bolometereintrittsfläche ausgefüllt wird. Die anderen Öffnungen sind von Bolzen mit vergoldeter Grundfläche, wie im Vordergrund abgebildet, verschlossen.

Reflexionsvermögen unbekannter Oberflächen, z.B. der Haut, kann direkt in Prozenten des Reflexionsvermögens einer hochglanzpolierten Goldfläche angegeben werden, das im Gebiet oberhalb $\lambda \approx 2\ \mu m$ nach Gier, Dunkle und Bevans[12], Brügel[11] sowie Pepperhoff[13] zwischen $r = 0.90$–0.98 liegt. Vergleiche des Reflexionsvermögens der Haut im Bereich ihrer Temperaturstrahlung gegen Magnesiumoxyd (Pepperhoff) oder gegen Carbonate (Schaefer, Bormuth und Matossi[14], Duval, Duval und Lecomte[15,16]) müssen zu erheblich überhöhten scheinbaren Werten führen, da sich solche Oberflächen im Ultrarotbereich auf Grund ihres sehr schlechten, bandenabhängigen und vom Alter der Schichten beeinflussten eigenen Reflexionsvermögens im Gegensatz zum sichtbaren Spektralgebiet nicht eignen.

Abb. 5 zeigt die zerlegte Halbkugel mit dem vergoldeten Vergleichsreflektor, einem Verschlussbolzen und dem eingeführten Bolometer.

In Abb. 6 sind die Ergebnisse der Reflexionsmessungen dargestellt. Sie bestätigen

Literatur S. 501

frühere Ergebnisse von Kuppenheim und Heer[17], die über ein schlechteres Reflexionsvermögen pigmentierter Hautpartien (Stirn) gegenüber unpigmentierten (Schulter) berichtet hatten. Es ergibt sich ferner die Aussage, dass die lebende menschliche Haut oberhalb $\lambda \approx 3.5\ \mu m$ nur noch ein Reflexionsvermögen von weniger als 0.5% aufweist. Der Spektralbereich unterhalb $\lambda \approx 3.5\ \mu m$ liefert aber nur eine anteilige Strahlung von etwa 0.1% der Gesamtemission, so dass dessen Verhalten vernachlässigt werden darf.

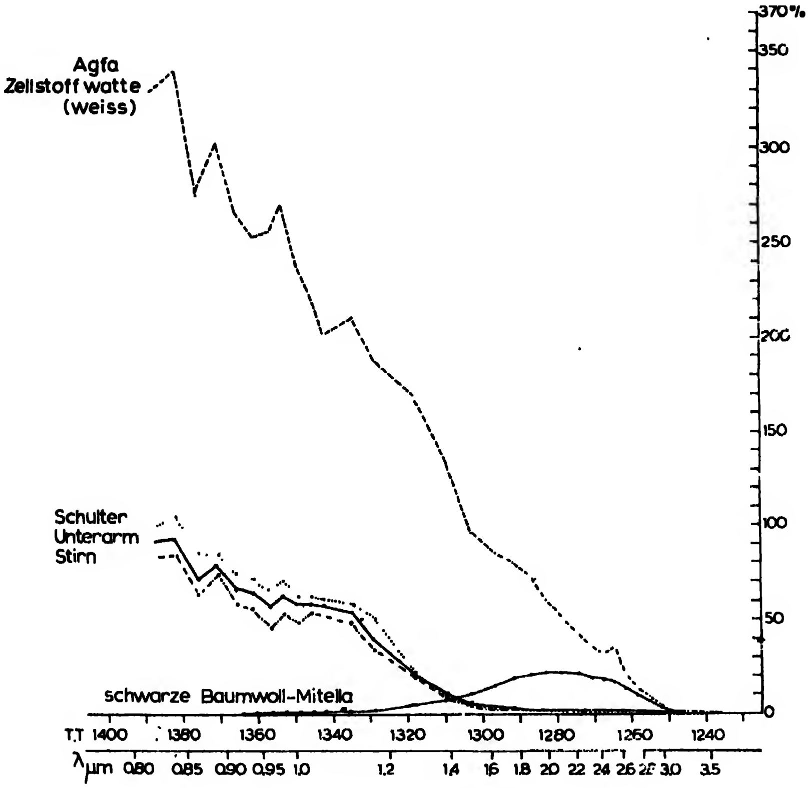

Abb. 6. Ergebnisse der Messungen des Reflexionsvermögens der lebenden menschlichen Haut, sowie von Zellstoffwatte und einer schwarzen Baumwoll-Mitella. Angaben in % des Reflexionsvermögens einer hochglanzpolierten Goldfläche.

Die Ergebnisse werden durch Untersuchungen über die Durchlässigkeit des Praeputium penis gestützt, die im nahen Ultrarot von den Absorptionsbanden des Wassers bestimmt wird und oberhalb $\lambda \approx 3\ \mu m$ nicht mehr nachgewiesen werden konnte.

Der Aufbau der Versuchsanordnung entsprach Abb. 4; anstelle der Halbkugel wurde hinter dem Austrittsspalt des Monochromators in etwa 1 cm Abstand das Bolometer in Stellung gebracht, vor dessen Eintrittsfläche sich unmittelbar die angespannte Hautfalte befand.

Zusammenfassend kann anhand der gut übereinstimmenden Ergebnisse aus Messungen des Emissions- und Reflexionsvermögens sowie der Durchlässigkeit der lebenden menschlichen Haut festgestellt werden, dass sie praktisch die Strahlungseigenschaften eines idealen schwarzen Körpers besitzt. Im nahen ultraroten Spektralbereich werden die Resultate durch frühere Untersuchungen von Clark, Vinegar und Hardy[18] gestützt. Ein Emissionsvermögen von $\varepsilon \geq 0.99$ berechtigt dazu, Temperatur-

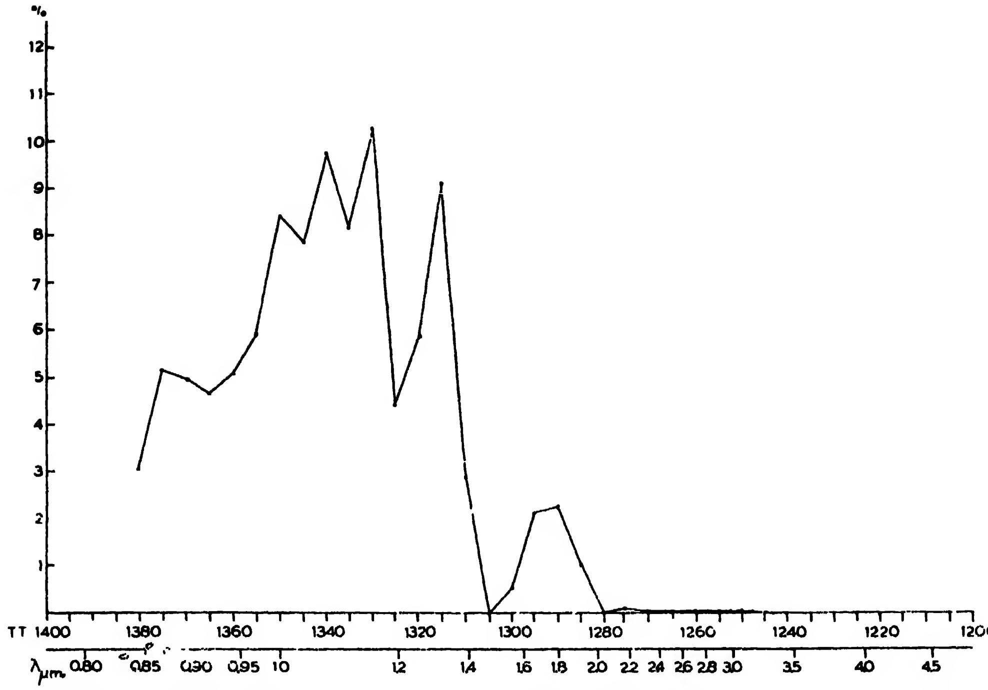

Abb. 7. Ergebnisse der Messungen der Durchlässigkeit der lebenden menschlichen Haut. (Praeputium unter Vermeidung des Durchstrahlens grösserer Blutgefässe). Angaben in % der auffallenden Strahlung.

bestimmungen der menschlichen Körperoberfläche auf radiometrischem Wege durchzuführen und die Vorteile solcher, mit modernen Aggregaten überaus schnellen Messmethoden auf ihre praktische Bedeutung für die Klinik zu überprüfen.

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1 W. FALTA, *Zeiss-Nachrichten*, (1956) 193.
2 L. NICOLAI, *Pflügers Arch. ges. Physiol.*, 229 (1931) 385.
3 L. NICOLAI, *Pflügers Arch. ges. Physiol.*, 229 (1931) 372.
4 E. LEHRER, *Z. techn. Physik*, 18 (1937) 393.
5 E. LEHRER, *Z. techn. Physik*, 23 (1942) 169.
6 M. CZERNY, W. KOFINK UND W. LIPPERT, *Ann. Physik* (6. Folge), 8 (1950) 65.
7 W. WOLTERSDORFF, *Z. Physik*, 91 (1934) 230.
8 M. CZERNY, *Physik. Z.*, 45 (1944) 207.
9 MECKE, VON ANGERER, MANNKOPFF UND SEIDEL, in LANDOLT-BÖRNSTEIN (Redaktör), *Zahlenwerte und Funktionen* ..., Band I, 2. und 3. Teil, Springer-Verlag, Berlin, 1951.
10 G. KORTÜM, *Kolorimetrie, Photometrie und Spektrometrie*, Springer-Verlag, Berlin, 1955.
11 W. BRÜGEL, *Physik und Technik der Ultrarotstrahlung*, Verlag Curt R. Vincentz, Hannover, 1951.
12 J. T. GIER, R. V. DUNKLE UND J. T. BEVANS, *J. Opt. Soc. Am.*, 44 (1954) 558.
13 W. PEPPERHOFF, *Temperaturstrahlung*, Verlag Dr. Dietrich Steinkopff, Darmstadt, 1956.
14 C. SCHAEFER, C. BORMUTH UND F. MATOSSI, *Z. Physik*, 39 (1926) 648.
15 R. DUVAL, C. DUVAL UND J. LECOMTE, *Compt. rend.*, 215 (1942) 525.
16 R. DUVAL, C. DUVAL UND J. LECOMTE, *Bull. soc. chim. France*, 10 (1943) 517.
17 H. F. KUPPENHEIM UND R. R. HEER, *J. Appl. Physiol.* 4 (1952) 800.
18 C. CLARK, R. VINEGAR UND J. D. HARDY, *J. Opt. Soc. Am.*, 43 (1953) 993.

Remissionsmessungen an menschlicher und tierischer Haut nach UV-Bestrahlung

Das Problem der Bestimmung von Veränderungen der Hautfarbe infolge einer bestimmten Behandlung interessiert schon seit Jahrzehnten eine Reihe von Forschern. In unserem Falle handelt es sich um die Verfolgung der Hautreaktion nach Bestrahlung mit ultraviolettem Licht. Erstmals gab Bode[1] hierfür eine quantitative Methodik an. Seither sind einige Berichtigungen erfolgt, besonders durch Edwards und Duntley[2] hinsichtlich der spektralen Hautremission. Am Grundprinzip hat sich aber nichts geändert. Es wird die Remission bei bestimmten Wellenlängen in Beziehung gesetzt, um quantitative Aussagen über den Anteil von Erythem und Pigmentierung an der UV-Reaktion zu erhalten. Vorwiegend wird hierbei die Remission zu einigen bestimmten Zeitpunkten ermittelt. Es besteht das Interesse, die Zahl der Messungen für jede Hautstelle soweit wie möglich zu reduzieren.

Die Befunde verschiedener Autoren gehen oft auseinander. Es hat auch nicht an kritischen Stimmen zur angegebenen Methodik gefehlt. Wir stellten uns die Aufgabe, mittels Remissionsmessungen den Ablauf der UV-Reaktion möglich exakt zu untersuchen, um die Bewertungsverfahren beurteilen zu können. Als Apparatur hierzu verwendeten wir ein Pulfrich-Photometer mit Ulbricht'scher Kugel und Metallinterferenzfiltern im Bereich zwischen 403 und 696 mμ.

Da an der menschlichen Haut die Bräunung bei der Untersuchung des Erythemablaufs stört, studierten wir diesen an der Kaninchenhaut. Die Beobachtungen an tierischer und menschlicher Haut stimmten in den Schlussfolgerungen überein. Im folgenden beschränken wir uns auf eine Zusammenfassung der wichtigsten Schlussfolgerungen aus unseren Untersuchungen.

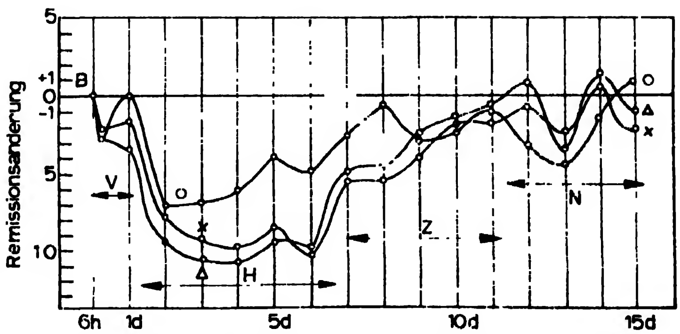

Abb. 1. Zeitliche Remissionsänderungen bestrahlter Kaninchenhaut. *B*-Tag der Bestrahlung; *L*-Latenz; *V*-Vorphase; mit Rotaufhellung; *H*-Hauptphase; *Z*-Zwischenphase; *N*-Nachphase (Durchblutungs- und Transparenzänderungen in Verhältnisse an normaler Haut übergehend). Messung bei 622 (o), 551 ($\triangle$) und 501 mμ (x). Ordinate: Differenz der Remission zum Zeitpunkt der Messung und vor der Bestrahlung.

(1) *Ablauf des Erythems*

Das Erythem besteht aus einer mehr oder weniger starken Dunklung und über-

lagerten Helligkeitsänderungen. An der Kaninchenhaut lief das Erythem in Phasen ab (Abb. 1). Der Phasenablauf konnte auch für die Reaktion an menschlicher Haut wahrscheinlich gemacht werden. Von einer bestimmten Dunklung im Grün an (die Höhe hängt von der Lokalisation bzw. Struktur der Hautstelle ab) beeinflusst das Erythem etwa proportional die Remission im Rot. Man sieht dies deutlich im Dia-

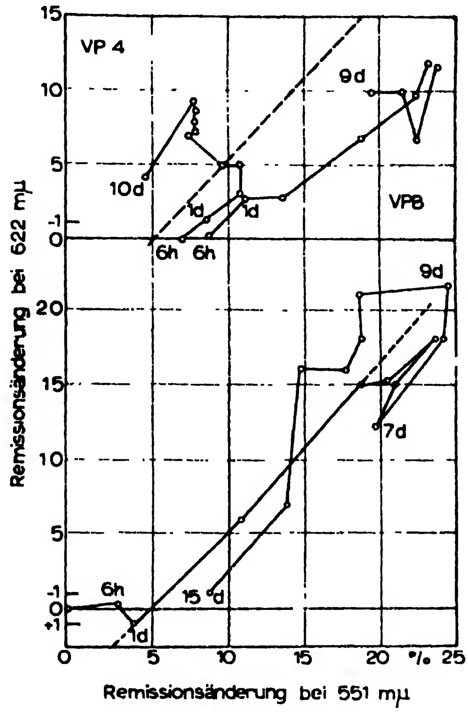

Abb. 2. Beziehung zwischen den Remissionsänderungen im Rot und Grün. Oben: menschliche Haut: VP 4 – bräunungsbetonte Reaktion, VP 8 – rötungsbetonte Reaktion. Unten: Kaninchenhaut — reines Erythem. An den Punkten Abstand der Messung von der Bestrahlung: h -Stunde; d Tag.

gramm der Abb. 2. Die Erscheinung tritt sowohl bei menschlicher als auch bei Kaninchenhaut auf. Der wellenförmige Ablauf des Erythems verlangt, dass zu seiner Beurteilung die Remission der Haut mehrere Tage nach der Bestrahlung mit mindestens einer täglichen Messung zu ermitteln ist. Das Erythem hält noch längere Zeit an unter der Bräunung und verrät sich durch die rötliche Tönung der letzteren.

(2) Die Pigmentierung

Der Beginn der Pigmentierung lässt sich aus den Veränderungen der Remission im Rot bestimmen, wenn der Einfluss des Erythems berücksichtigt wird. Sehr häufig fiel mit dem derart ermittelten Beginn der Pigmentierung eine Aufhellung im Rot zusammen. Wir halten diese „Pigmentaufhellung" für ein Anzeichen des Entstehens stärker remittierender Vorprodukte des Melanins. Die Remission im Grün wird durch die Pigmentvermehrung beeinflusst, sobald die Absorption der Pigmentgranula die der blutgefüllten Gefässe übersteigt. Die Zunahme des Pigmentes erfolgt unregelmässig.

Literatur S. 505

Sie geht teilweise stufenförmig vor sich. Dorno[3] bezeichnete eine derartige Erscheinung als Pigmentschub.

(3) *Einfluss von Transparenzänderungen*

Bis jetzt wird bei der Beurteilung der Hautreaktion nach UV-Licht-Bestrahlung nur das Erythem und die Pigmentierung berücksichtigt. Wir fanden aber auch einen wesentlichen Einfluss von Transparenzänderungen der oberen und mittleren Hautschichten. Sie haben offensichtlich grossen Anteil an dem wellenförmigen Ablauf des Erythems. Bereits an unbestrahlter Haut fanden wir beträchtliche Helligkeitsschwankungen als Folge von Transparenzänderungen.

Bei einigen Hautreaktionen fanden wir als Folge veränderter Transparenz besonders starke Dunklungen. Dieser Effekt wurde durch starkes subcutanes Fettgewebe noch verstärkt und macht die Beurteilung der UV-Reaktion nach dem Rötungsgrad fragwürdig.

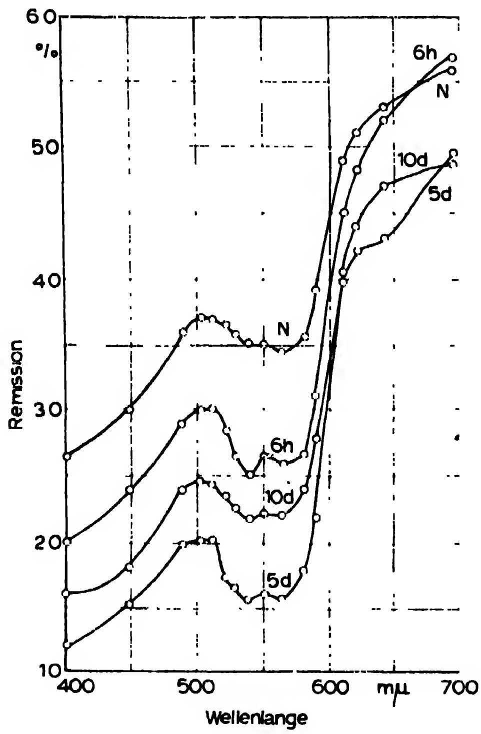

Abb. 3. Spektrale Remissionskurven einer menschlichen Haut (Unterarm) zu verschiedenen Zeitpunkten vor (*N*) und nach der Bestrahlung.

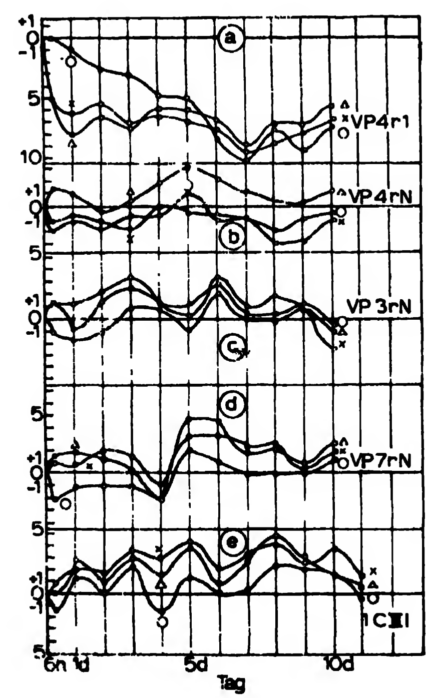

Abb. 4. Remissionsänderungen menschlicher und tierischer (*e*) Haut abhängig von der Zeit *a* — bestrahlt; *b e* unbestrahlt.

(4) *Vereinfachte spektrale Messungen*

Unsere Untersuchungen zeigten, dass es durchaus vertretbar ist, die Remissionsänderungen bei wenigen Wellenlängen zu messen und zur Beurteilung der Hautreaktion heranzuziehen. Als Voraussetzung hierfür muss aber die spektrale Remissionskurve bekannt sein. Wir fanden die spektrale Remissionskurve der Haut von Mensch und Kaninchen so, wie sie von Edwards und Duntley beschrieben worden ist. Ein

Maximum im Rot bei 630 mμ wie Bode fanden wir nicht. Die Verschiebung der Remissionskurven während der Hautreaktion entspricht aber in grossen Zügen den Vorstellungen seit Bode (Abb. 3). Für die vereinfachte Darstellung der Remissionsänderungen wählten wir als Minimum drei Wellenlängen aus. Es sind dies 504, 551 und 622 mμ. Sie liegen an markanten Punkten der Remissionskurve und lassen Vergleiche mit den Befunden anderer Autoren zu.

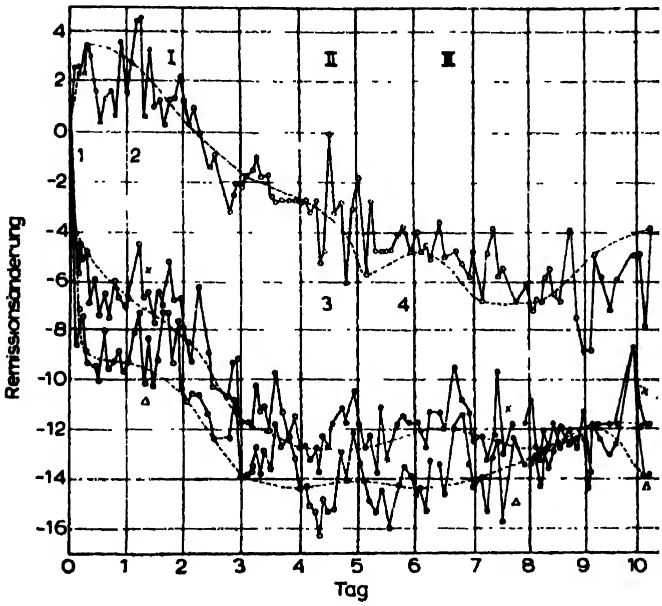

Abb 5. Remissionsänderungen menschlicher Haut abhängig von der Zeit nach Bestrahlung mit UV-Messungen im Abstand von 2 bis 6 Stunden. Vergl. mit Abb. 1 und 4.

An der unbestrahlten Haut fanden wir teilweise grosse Remissionsänderungen (Abb. 4). Sie sind oft durch Transparenzänderungen bedingt. Da diese Helligkeitsänderungen an benachbarten Hautstellen unterschiedlich ablaufen, halten wir einen direkten Vergleich einer unbestrahlten Hautstelle mit der bestrahlten für unzulässig. Wir tragen die Differenz der Remission in unseren Kurven auf, die zwischen Zeitpunkt der Messung und vor der Bestrahlung besteht. Als wir die Remissionsänderungen im Grün und Blau in einem Diagramm in Beziehung setzten (Abb. 2), erhielten wir für rötungs- und bräunungsbetonte Reaktionen deutlich unterschiedliche Kurven. Erstere glichen den von reinen Erythemen an Kaninchenhaut erhaltenen.

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[1] H. G. BODE, *Strahlentherapie*, 51 (1934) 81.
[2] E. A. EDWARDS UND S. Q. DUNTLEY, *Am. J. Anat.*, 65 (1939) 1.
[3] C. DORNO, *Strahlentherapie*, 22 (1926) 70.

Quantitative measurements of the reactions of human skin to ultraviolet light

Sunburn and tanning are commonly observed reactions of human skin to ultraviolet radiation. These have frequently been described in a clinical manner, most often on the basis of a minimal perceptible erythema. Our studies of the effects of ultraviolet light on human skin have included a large number of instrumental measurements of erythema and of melanin which make possible much more complete evaluations of the operating characteristics of these responses.

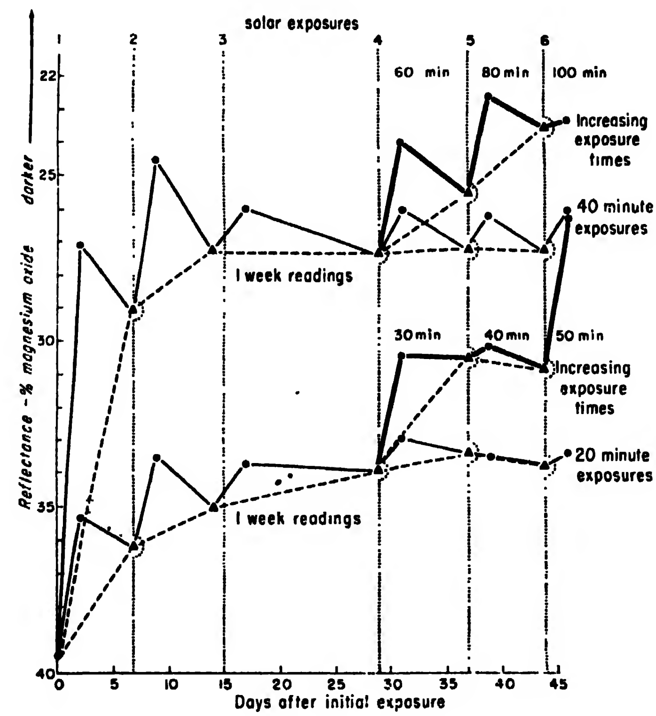

Fig. 1. Time course of mean green reflectance readings with multiple solar exposures.

Photoelectric reflectometers[1,2] permit expression of skin color in numerical units, and provide some of the information supplied by more expensive reflectance spectrophotometers. We have used the Photovolt 610-T reflectometer[3] with blue, green and amber tristimulus filters and also with a red glass filter (Corning 2403). Since the principal aim is to measure melanin and hemoglobin separately, narrow band pass interference filters have also been used to accomplish abridged spectrophotometry. An interference filter with transmittance peak at 542 or 576 mμ or the green tristimulus filter is used for response to hemoglobin plus melanin, and the red filter to measure melanin with minimal effect of hemoglobin[4,5].

In general, clinical findings are confirmed, but the reflectance numbers give quanti-

tative validity and permit studies of correlation and regression[4]. These studies have been oriented particularly to the effects of sunburn produced by sunlight or by a high-pressure mercury arc filtered through a narrow band pass interference filter with a maximum at 295 mμ, and to photosensitized reactions produced in human skin by oral or topical administration of 8-methoxypsoralen plus sunlight or long-wave ultraviolet light from black light fluorescent lamps.

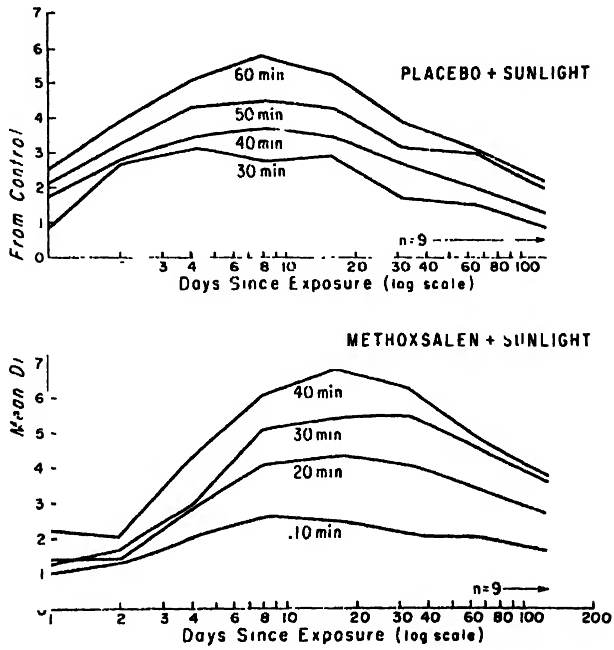

Fig. 2. Time course of melanin following solar exposure. Red filter reflectance, n = 10, except where indicated.

The psoralen (furocoumarin) compounds are a series of materials found in plants where they apparently function as growth and germination inhibitors[6]. The native plants or refined preparations cause phytophotodermatitis. These compounds have also had a vogue for the treatment of vitiligo and for the potentiation of skin pigmentation in persons genetically maladapted to sunny climates, and in persons who value suntan.

Fig. 1 shows the time course of reflectance readings in 30 men who had repetitive noon-day sun exposures of 20 and 40 min at latitude 45°N starting the second week of July. The successive exposures are indicated by the dotted vertical lines. This study was done in a prison and it was not possible administratively to obtain all readings on all the men. Hence the means are based on 18 to 28 subjects. The readings just prior to the fourth exposure were taken two weeks rather than one week after exposure. The readings taken one week after exposure are connected by a dotted line. This line as confirmed by red filter measurements represents the melanin response. The steep rise to 2 days represents erythema for each exposure level. Equal weekly re-exposure times maintained a fixed melanin level. When the repetitive 40-min area was then exposed for progressive additional exposures to 60 and 80 min, an apparently linear

increase in melanin resulted. A similar but less conclusive result was obtained with the 20-min areas increased to 30 and 40 min. The two-day periods show progressive damping as equal exposures are repeated and then increase in erythema again with the increased exposure time, exhibiting the properties of a well-regulated system. The practical importance of progressive increase in exposure to promote tanning and solar tolerance is evident.

The time courses of melanin following single exposures of different durations are summarized in Fig. 2. Here mean values for red reflectance of 10 men exposed to sunlight for 30, 40, 50 and 60 min are compared with the same men exposed in a cross-over design to 10, 20, 30 and 40 min of sunlight 2 h after ingesting 30 mg of 8-methoxypsoralen. The photosensitizing effect of the 8MP is indicated by a greater pigment response and a much longer time required to reach the peak of melanization.

The red reflectance change at one and two days is largely the result of the slight sensitivity of this filter to the intense erythema present at that time. Comparison of the two erythemas gave significant correlations of 0.79 for 40 min ($y = 1.09 + 0.94x$). Thus subjects who developed more erythema with sunlight tended to develop more of a response to photosensitization.

The difference in response with and without psoralen depends on the ratio of 2900 to 3100 Å light which produces the sunburn reaction and the wavelengths around 3600 Å which produce the photosensitized reaction. Under the conditions of this experiment the two-day mean green reflectance difference from control for placebo + sunlight were 30 min, 7; 40 min, 10; 50 min, 11.7 and 60 min, 13.3. The methoxsalen areas showed for 10 min, 4; 20 min, 7; 30 min, 8.7 and 40 min, 11.3. Thus the erythema produced by photosensitization added the approximate equivalent of 10 min of solar exposure. The pigment response, however, was much greater, as shown in Fig. 2. For equal pigment response only about half as much exposure time was required. This was further analyzed by graphing and calculating the linear regressions of pigment response (red filter) on erythema (573-mμ filter). For sunburn the correlation was 0.76 ($y = 1.84 + 0.19x$), and for the photosensitized reaction the correlation was 0.67 ($y = -0.43 + 0.64x$). The scatter was much greater for the photosensitized response.

These studies of the operating characteristics of two types of ultraviolet damage to human skin do not give information on primary or delayed biochemical events but do indicate the response parameters into which the complex series of events must be fitted.

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[1] H. TRONNIER, *Strahlentherapie*, 104 (1957) 146.
[2] N. A. BARNICOT, *Human Biol.*, 30 (1958) 150.
[3] M. M. C. LEE AND G. W. LASKER, *Human Biol.*, 31 (1959) 252.
[4] F. DANIELS JR. AND J. D. IMBRIE, *J. Invest. Dermatol.*, 30 (1958) 295.
[5] F. DANIELS JR. AND C. E. HOPKINS *et al.*, *J. Invest. Dermatol.*, 32 (1959) 321.
[6] W. L. FOWLKS, *J. Invest. Dermatol.*, 32 (1959) 249.

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In vitro - Untersuchungen zu pathologischen Lichtreaktionen der Haut

Im Rahmen der Lichtdermatosen spielen die Photoallergien eine Rolle, die ihre nähere Untersuchung nicht nur für die Photopathologie, sondern auch für die Allergologie besonders lohnend erscheinen lässt. Im Prinzip handelt es sich bei den photoallergischen Phänomenen um den gleichen Vorgang wie bei den einfachen Allergien, mit dem Unterschied, dass das Allergen erst in der Haut unter Mitwirkung des Lichtes entsteht.

TABELLE I

ABLAUF DER ALLERGENBILDUNG BEI DER EINFACHEN UND DER PHOTOALLERGIE

	Umwelt (oder Körperinneres)	*Haut*		
		Umwandlung	*Eiweissbindung*	
Cutane Allergie I. Ordnung	Hapten – z.B. *p*-Aminobenzoesäure			→ Allergen PAB-Protein
Cutane Allergie II. Ordnung	Prohapten z.B. *p*-Aminobenzoesäureester	durch Hautfermente → (z.B. Verseifung)	Hapten *p*-Aminobenzoesäure	→ Allergen PAB-Protein
Photoallergie	Prohapten z.B. Sulfanilamid	durch Lichtwirkung → (z.B. Oxydation)	Hapten *p*-Hydroxylaminobenzolsulfonamid	→ Allergen HBSA-Protein

Während im einfachsten Falle der gewöhnlichen Allergie die Substanz durch die Haut als Hapten permeiert, sich dort mit Eiweiss zum eigentlichen Allergen umsetzt und in dieser Form mit früher gebildeten Antikörpern reagiert, ist für viele sog. Allergene zunächst eine Umsetzung, z.B. Verseifung, in das eigentliche Hapten und dann erst Allergen-Bildung und Reaktion mit dem Antikörper anzunehmen. Beispiele hierfür sind die *p*-Aminobenzoesäure einerseits, ihre Ester andererseits.

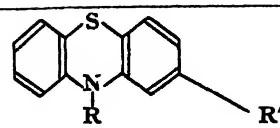
Ganz entsprechend permeiert im Falle der Photoallergie ein wirkungsloses Prohapten, das unter Lichteinwirkung in der Haut durch eine photochemische Umwandlung vor oder nach der Eiweissbindung in das eigentliche Allergen überführt wird. Neben der Frage, ob die photochemische Umwandlung vor oder nach der Eiweissbindung eintritt, interessiert hierbei naturgemäss besonders der Ablauf einer solchen photochemischen Reaktion in der Haut.

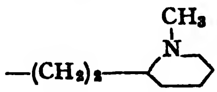
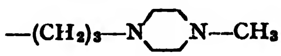
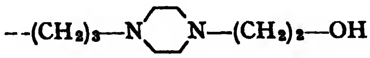
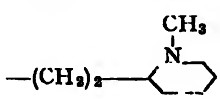
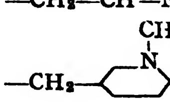
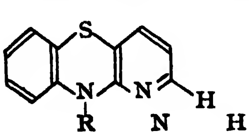
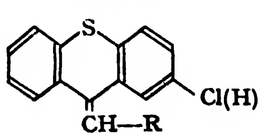
Dazu wurden unter den verschiedenen, photoallergisch wirksamen Substanzen die Sulfonamide und Phenothiazine näher auf ihr Verhalten gegen Licht *in vitro* untersucht.

Vertreter beider Verbindungsklassen zeigten dabei eine mehr oder weniger intensive Verfärbung im Licht, wobei die Phenothiazine je nach dem pH verschiedene, teils rote, teils blaue Farbtönungen aufwiesen. Auch bei einigen Sulfonamiden traten beim

Literatur S. 514

TABELLE II



Präparat	R	R'	*Blaufärbung beim Belichten*	*Photoallergie*
Chlorpromazin (Megaphen, Largactil)	$-(CH_2)_3-N(CH_3)_2$	Cl	+++	+
NP 207		Cl	++	+
6140 RP (Compazine)		Cl	++	möglich
Perphenazin (Trilafon, Decentan)		Cl	++	+
Thiopropazate (Dartal)		Cl	+++	sehr wahrscheinlich
7447 RP	$-(CH_2)_3-N(CH_3)_2$	OH	+++	dto.
7448 RP	dto.	OAc	++	möglich
4692 RP	$-CH_2-CH(CH_3)-N(CH_3)_2$	Br	++	dto.
6844 RP	$-CH_2-CH(CH_3)-CH_2-N(CH_3)_2$	F	(+)	ø
4627 RP	$-(CH_2)_3-N(CH_3)_2$	CH_3	ø	ø
6489 RP	dto.	C_2H_5	ø	ø
7210 RP	dto.	CN	ø	ø
1522 CB (Acepromazin)	dto.	$CO-CH_3$	ø	ø
8218 RP	dto.	$SO_2-N(CH_3)_2$	(+)	ø
4632 RP (Methopromazin)	dto.	OCH_3	(+)	ø
7044 RP (Methoxytrimeprazin)	$-CH_2-CH(CH_3)-CH_2-N(CH_3)_2$	OCH_3	ø	ø
Thioridazin (Melleril)		SCH_3	ø	ø
Promethazin (Atosil, Phenergan)	$-CH_2-CH(CH_3)-N(CH_3)_2$	H	ø	?
Mepazine (Pacatal)		H	ø	ø
Phenothiazin	H	H	ø	ø
3-Chlorphenothiazin	H	Cl	ø	ø
N-Methyl-3-chlorphenothiazin	$-CH_3$	Cl	ø	ø
N-Aethyl-3-chlorphenothiazin	$-CH_2-CH_3$	Cl	ø	ø
 und  4-Azaphenothiazine, Thiaxanthene			ø	ø

Belichten blaurote Färbungen auf, die bereits 1938 von Ottenberg, Fox und Cline[1,2] beobachtet worden waren. Solche photochemisch verfärbten Lösungen z.B. von Chlorpromazin ergaben nun bei entsprechenden Photoallergikern stark positive Hautreaktionen ohne zusätzliche Belichtung, während unverändertes Chlorpromazin nur nach zusätzlicher Belichtung der Haut zu Reaktionen führte. Dabei variierte die Intensität der Hautreaktion erheblich in Abhängigkeit von dem pH-Wert, bei dem die Vorbestrahlung der Lösung durchgeführt worden war. Und zwar war die Hautreaktion nach Vorbestrahlung bei pH-Werten um 5 am stärksten, während bestrahlte Lösungen mit einem pH von 6.5 oder 7.4 eine wesentlich schwächere Hautreaktion auslösten. Entsprechende Differenzen wurden nun auch im Verhalten der Chlorpromazin-Lösungen beim Belichten beobachtet. Während nämlich in annähernd neutra-

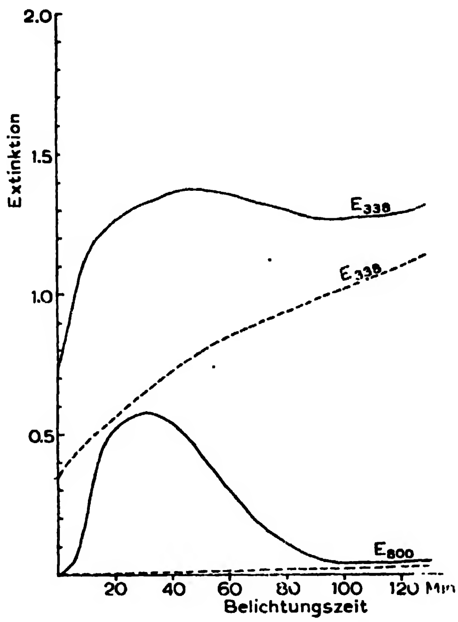


Abb. 1. Extinktion bei 338 mμ und 800 mμ nach Belichtung von — — Chlorpromazin 10^{-4} und - - - - - Promethazin 10^{-4} in Abhängigkeit von der Belichtungszeit.

ler Lösung rötliche Verfärbungen auftraten, bildete sich bei pH 4–5.6 aus Chlorpromazin beim Belichten ein allmählich aus gelber Lösung ausflockender, blauer Farbstoff.

Dass gerade dieser photochemischen Umwandlung eine besondere Bedeutung für die Photoallergie zukommen muss, ergibt sich nicht nur aus der starken Hautreaktion beim Photoallergiker auf solche blauen Lösungen, sondern auch aus dem Verhalten anderer Phenothiazine. Es zeigte sich nämlich, dass nur wenige Phenothiazin-Derivate unter diesen Bedingungen eine Blaufärbung entwickeln. Und zwar sind es gerade diejenigen, von denen sichere Photoallergien bekannt sind.

Neben dem Chlorpromazin sind dies das Perphenazin und das NP 207. Ausser diesen bildeten auch noch einige weitere Phenothiazine blaue Farbstoffe von denen bisher keine Photoallergien beschrieben worden sind.

Die grössere Gruppe der untersuchten Substanzen enthielt jene Phenothiazine, die

Literatur S. 514

beim Belichten keine Blaufärbung zeigten und von denen bisher keine sicher.. Photoallergien beschrieben worden sind. Bei diesen Substanzen waren auch unbe lichtete Läppchenteste bei Allergikern positiv.

Ähnliche — in chemisch-struktureller Hinsicht allerdings weniger übersichtliche — Verhältnisse wurden auch bei den Sulfonamiden beobachtet. Auch hier zeigten die-

TABELLE III

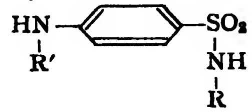
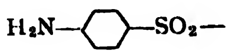
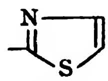
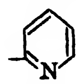
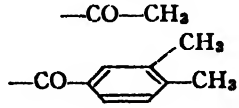
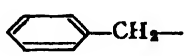
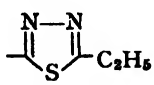
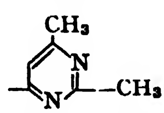
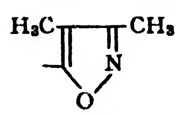
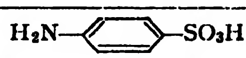
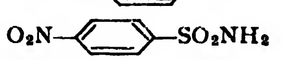
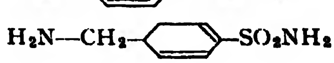
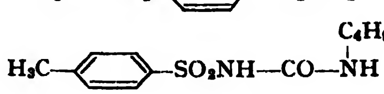
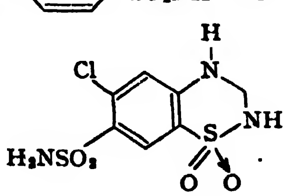
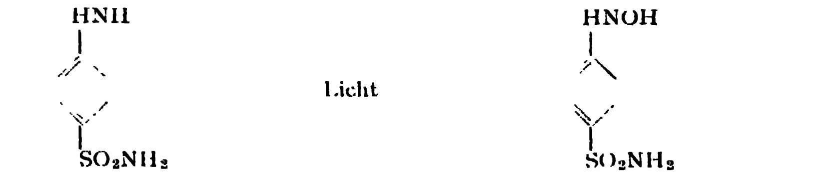
Präparat			*Verfärbung im Licht*	*Photoallergie*
Sulfanilamid	H	H	++++	+
(Neo)-Uliron		—CH_3	+++	+
Sulfathiazol	H		+++	+
Sulfapyridin	H			+
BZ 55	H	—CO—NH—C_4H_9	++	+
Sulfacetamid	H	—CO—CH_3		+
Irgafen	H			+
Septazine		H		+
Globucid	H		(+)	ø
Badional	H	—CS—NH_2	ø	ø
Aristamid	H		ø	ø
Gantrisin	H		ø	ø
Sulfanilsäure			+	ø
p-Nitrobenzolsulfonamid			ø	ø
Marfanil			ø	ø
D 860			+	ø
Esidrix			(+)	ø

TABELLE IV

CHEMIE DER PHOTOALLERGENE

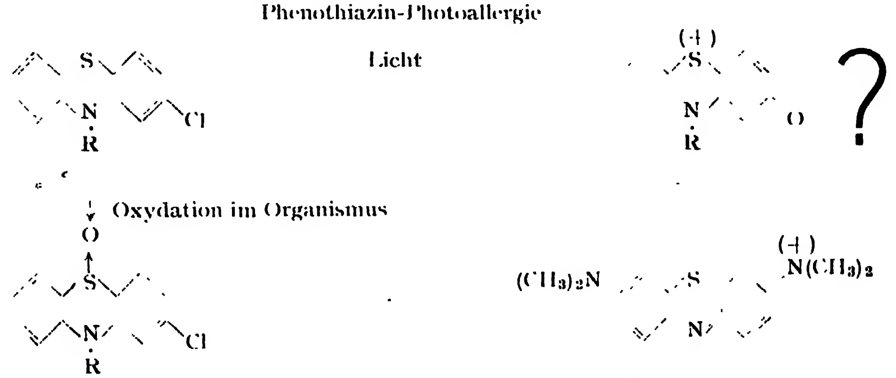


p-Aminobenzolsulfonamid — *p*-Hydroxylaminobenzolsulfanomid (Schwarz, Speck, Burckhardt) (Shinn 1939)

Sulfonamid-Photoallergie

Epicutanteste		
ohne Licht	o	+
mit Licht	+	+

Phenothiazin-Photoallergie



Sulfoxyd — Methylenblau

Epicutanteste bei Photoallergikern negativ

jenigen Substanzen die stärkste Verfärbung beim Belichten, die als Photoallergene bekannt sind.

Während bei den Sulfonamiden durch die Arbeiten von Schwarz, Speck und Burckhardt[3-6] bereits die entscheidende Zwischensubstanz in Gestalt des erstmals 1939 von Shinn[7] u.a. beim Belichten von Sulfanilamid erhaltenen *p*-Hydroxylaminobenzolsulfonamids bekannt ist, gelang es bisher nicht, die chemische Natur der aus den Phenothiazinen entstehenden, photochemischen Umwandlungsprodukte aufzuklären. Diese Substanzen sind nicht nur sehr unbeständig, sondern waren bisher auch nur mit sehr schlechter Ausbeute präparativ gewinnbar. Aus allen bisherigen Befunden lässt sich aber sagen, dass Sulfoxyde offenbar keine Rolle spielen, dass vielmehr einer Umwandlung in der 3-Stellung die grösste Bedeutung zukommt. Und zwar wird, wie sich aus Infrarot-Spektren ergab, offenbar das Chlor abgespalten und durch einen Sauerstoff-haltigen Substituenten ersetzt. Diese Annahme wird durch ein entsprechendes Verhalten der 3-Hydroxyverbindung ganz wesentlich gestützt.

Die bei diesen Untersuchungen festgestellte Parallelität zwischen photoallergisierender Wirkung und photochemischem Verhalten, dessen pH-Abhängigkeit im Falle des Chlorpromazins auch die Tatsache erklären dürfte, dass nur die percutane Aufnahme zu Photoallergien führt, erscheint in zweierlei Hinsicht bedeutsam.

Literatur S. 514

TABELLE V

PATHOGENESE DER PHOTOAUTO- UND PHOTOHETEROREAKTION

	Photoautoreaktion	*Photoheteroreaktion*
Wirksamer Lichtbereich	UVB (bei Photosensibilisierung auch andere Bereiche)	verschieden
Reaktionssystem	*Physiologische* Hautbestandteile	exogene oder endogene *unphysiologische* Hautbestandteile
Reaktion	photochemische Umwandlung (durch Photosensibilisierung verstärkt)	photochemische Umwandlung
Effekt	Lichterythem usw. (durch Photosensibilisierung verstärkt)	von der Natur der photochemisch entstandenen Produkte abhängig

Einmal lässt die photochemische Untersuchung neuer Substanzen eine gewisse Vorhersage photoallergischer Nebenwirkungen zu, zum anderen weist die Feststellung photochemischer Prozesse in der Haut auf deren Bedeutung im Rahmen der gesamten Photopathologie.

Die grosse Zahl klinischer und experimenteller Beobachtungen mit photodynamisch wirksamen, photosensibilisierenden Substanzen hat das Interesse vielleicht etwas einseitig in diese Richtung gelenkt, auf Hautreaktionen also, die sich als Photoautoreaktionen an dem physiologischen, autochthonen, photochemischen Reaktionssystem der Haut abspielen. Wie die Photoallergie und ihre Pathogenese aber zeigen, sollte daneben auch mit Photoheteroreaktionen gerechnet werden, mit Reaktionen also, bei denen normalerweise in der Haut nicht vorkommende Stoffe photochemisch in pathogene Substanzen umgewandelt werden und dadurch zu Hautveränderungen führen, die sich im Gegensatz zur Photosensibilisierung morphologisch mehr oder weniger weitgehend von der physiologischen Lichtreaktion der Haut unterscheiden.

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1 R. OTTENBERG UND C. L. FOX, *Proc. Soc. Exptl. Biol. Med.*, 38 (1938) 479.
2 C. L. FOX, J. E. CLINE UND R. OTTENBERG, *J. Pharmacol. Exptl. Therap.*, 66 (1939) 99.
3 K. SCHWARZ UND M. SPECK, *Dermatologica*, 114 (1957) 232.
4 W. BURCKHARDT, *Dermatologica*, 83 (1941) 63.
5 W. BURCKHARDT, K. SCHWARZ UND M. SCHWARZ-SPECK, *Schweiz. med. Wochschr.*, (1957) 954.
6 W. BURCKHARDT, *Akt. Probleme Dermatol.*, 1 (1959) 301.
7 L. SHINN, E. R. MAIN UND R. R. MELLON, *Proc. Soc. Exptl. Biol. Med.*, 52 (1939) 736.
8 S EPSTEIN, *J. Invest. Dermatol.*, 2 (1939) 43.
9 S. EPSTEIN UND R. J. ROWE, *J. Invest. Dermatol.*, 29 (1957) 319.
10 S. EPSTEIN, *AMA. Arch. Dermatol.*, 81 (1960) 175.
11 H. IPPEN, *2. Intern. Photobiol. Kongr.*, 1957, p. 73.
12 H. IPPEN, *Klin. Wochschr.*, 36 (1958) 587.
13 H. IPPEN, *Berufsdermatosen*, 7 (1959) 12.
14 H. IPPEN, *Arch. klin. exptl. Dermatol.*, 211 (1960) 261
15 H. IPPEN, *Strahlentherapie*, 112 (1960) 234.
16 K. H. SCHULZ, A. WISKEMANN UND K WULF, *Arch. klin. exptl. Dermatol.*, 202 (1956) 285.

Tierexperimentelle Untersuchungen zur Keratokonjunktivitis photoallergica

Das 1957 beschriebene Krankheitsbild der Keratokonjunktivitis photoallergica (K.ph.) ist charakterisiert durch pathologische Reaktionen von Hornhaut und Bindehaut auf langwelliges Ultraviolettlicht (UVA). Dieser Strahlenbereich des Sonnenlichtes bewirkt normalerweise die direkte Pigmentierung und ist als auslösender Spektralbereich bei photodynamischen Hautreaktionen, z.B. durch Phenothiazine bekannt.

Die entzündlichen Veränderungen am Auge können dabei derart excessiv sein, dass es zur Perforation der Hornhaut kommt. In einem Beobachtungsfall wusste man sich nicht anders zu helfen, als dem Patienten monatelang beide Augen bis auf einen schmalen Spalt zu vernähen.

Wie kommt es nun zu diesem eindrucksvollen Krankheitsbild? Pathologische Hautreaktionen auf langwelliges Ultraviolettlicht kennt man in der Dermatologie bei polymorph Lichtkranken im Sinne Haxthausens und bei Menschen, die durch exogen zugeführte photodynamisch wirksame Substanzen gegen Licht sensibilisiert wurden.

In der Veterinärmedizin sahen Enzie und Whitmore[1] bei der oralen Phenothiazin-Wurmbehandlung von Ziegen mit 25 g bei ausgewachsenen Tieren und 12.5 g bei Jungtieren das Auftreten einer Keratokonjunktivitis 42 Stunden nach Medikamentanwendung bei sonnigem Wetter.

Alle bisher beobachteten, an einer K.ph. erkrankten Menschen hatten Umgang mit photodynamisch wirksamen Substanzen oder bekamen derartige Stoffe aus therapeutischen Gründen zugeführt. Es handelte sich dabei vorwiegend um Sulfonamide, Paraaminosalicylsaure und Phenothiazine. Die hypothetische Annahme einer Lichtsensibilisierung durch solche Substanzen lag deshalb nahe.

Kann man bei Tieren mit solchen Stoffen ähnliche oder gleiche Krankheitsbilder experimentell hervorrufen? Der Klärung dieser Frage dienten umfangreiche Tierexperimente mit unterschiedlichen photodynamisch wirksamen Substanzen in verschiedener Weise an verschiedenen Tierarten appliziert.

Den grundsätzlichen Beweis dafür, dass es möglich ist, durch lokale Anwendung einer photodynamisch wirksamen Substanz am Auge eine Lichtsensibilisierung hervorzurufen, führten wir mit einer 2%igen Lösung von N-(3'-Dimethylamino-propyl)-3-chlorphenothiazin PH7 am Auge von Wistarratten[4]. Nach 15-maliger Applikation innerhalb von 40 Tagen von je 5–6 Tropfen dieser Lösung in den Konjunktivalsack der Tiere und anschliessender Sonnenbelichtung kam es zur Entwicklung einer Keratokonjunktivitis mit erheblicher Lichtscheu und z.T. bleibenden Veränderungen an Hornhaut und Konjunktiva, wie Sie sie auf folgenden Bildern sehen. Im Dunkeln belassene Kontrolltiere blieben erscheinungsfrei.

Die tiefgreifenden Veränderungen an den Augen der Albinoratten lassen sich am besten mit Hilfe des Spaltlampenmikroskopes nachweisen, sind jedoch wegen der Pigmentarmut der Augen auf Photogrammen nur sehr schwer festzuhalten.

Bei den Kontrolltieren (Abb. 1) erkennen Sie die perlucide Hornhaut, die den Blick auf die Regenbogenhaut freigibt. Diese ist praktisch pigmentlos, sodass ihre Farbe weitgehend durch das Blut der Irisgefässe bestimmt wird.

Literatur S. 518

Nach der Lichtsensibilisierung kommt es zunächst zu einer akuten, mit einer Verborkung der Lidränder einhergehenden Konjunktivitis (Abb. 2). Wenige Tage darauf stellt sich dann eine Epithelnekrose der Hornhaut sowie eine oberflächliche Parenchymtrübung ein, die allmählich auf die mittleren Hornhautlagen übergreift. Am Ende des Versuches bestehen in der Lidspaltenzone dichte Opazitäten der Cornea

Abb. 1. Normales Rattenauge.

Abb. 2. Mit Chlorphenothiazin-Tropfen lichtsensibilisiertes Rattenauge (Frühstadium).

Abb. 3 und 4. Lichtsensibilisierte Rattenaugen (Spätstadium).

mit oberflächlicher Gefässeinsprossung (Abb. 3 und 4). In zwei Fällen kam es sogar zu einer Hornhauteinschmelzung mit Irisvorfall und Ausheilung in Form eines Leucoma adhaerens.

Zur Klärung der Frage, ob die Lichtsensibilisierung abhängig ist von der Menge der applizierten photodynamisch wirksamen Substanz und der Lichtintensität wurden ergänzende Versuche mit 10%iger gleichartiger Phenothiazinlösung bei 20 Wistarratten angesetzt. 5–6 Tropfen dieser Lösung wurden den Tieren in 2–3 tägigen Abständen in den Konjunktivalsack geträufelt, anschliessend wurden 10 Tiere ins

Dunkle gebracht, die anderen 10 für 2–3 Stunden dem Herbstsonnenlicht ausgesetzt. Schon nach der 3. spätestens 4. Behandlung mit Bestrahlung kam es bei den Belichtungstieren zur Ausbildung einer Keratokonjunktivitis, wie wir sie früher mit der 2%igen Lösung erst nach 15 Behandlungen erzielen konnten. Obgleich auch die

Abb. 5. Feingeweblicher Befund beim normalen Rattenauge. Färbung. Azan nach Heidenhain.

Abb. 6. Feingeweblicher Befund beim lichtsensibilisierten Rattenauge. Trichromfärbung nach Masson.

Dunkeltiere — wohl toxisch bedingte — Reizungen der Augen aufwiesen, ist eindeutig feststellbar: Die Menge der applizierten photodynamischen Substanz beschleunigt die Lichtsensibilisierung des Tierauges ganz wesentlich.

Zur Beurteilung, ob die Strahlenintensität für die Entwicklung der Keratokonjunktivitis von Bedeutung ist, wurden bei allen Versuchen die Bestrahlungseffekte von Tagen mit bedecktem Himmel mit denen von wolkenlosem Himmel verglichen. Hierbei war eindeutig feststellbar, dass stärkere Lichtintensität zu stärkeren klinischen Symptomen führte.

Die Frage, ob durch intensivere Bestrahlung eine schnellere Lichtsensibilisierung der Augen bewirkt wurde, konnte mit unserer Versuchsanordnung nicht sicher beantwortet werden, erscheint aber wahrscheinlich.

Welche feingeweblichen Veränderungen bietet nun ein derart gegen Licht sensibilisiertes Rattenauge im Vergleich zu dem Befund eines Kontrolltieres?

Auf der nächsten Aufnahme erkennen Sie einen Horizontalschnitt durch Hornhaut und Kammerwinkel des Auges eines unbestrahlten Tieres (Abb. 5). Epithel und Glasmembranen sind intakt, das Parenchym der Cornea selbst zeigt eine normale Lamellenstruktur ohne jede entzündliche Infiltration oder Gefässeinsprossung. Beim

Literatur S. 518

bestrahlten Tier (Abb. 6) sind die oberen 2/3 der Cornea aufgelöst durch ein Granulationsgewebe, welches sich aus Fibroblasten, kleinrundzelligen Elementen und zahlreichen neugebildeten Kapillaren aufbaut. Lediglich die hinteren Membrananteile sind in den Prozess nicht einbezogen. Bei 2 Fällen fand sich auch histologisch ein Leukoma adhaerens nach Perforation eines durch die Photosensibilisierung gesetzter Hornhautulcus.

Abschliessend möchten wir feststellen: Direkt am Rattenauge applizierte photodynamisch wirksame Substanzen können zur lokalen Lichtsensibilisierung dieses Organs führen. Die Schnelligkeit mit der die Lichtsensibilisierung eintritt, dürfte abhängig sein von der Menge der applizierten photodynamisch wirksamen Substanz und wahrscheinlich auch von der Lichtintensität.

Als typische feingewebliche Veränderung des lichtsensibilisierten Rattenauges findet man im Frühstadium eine Nekrobiose des Epithels, einen Zerfall der oberflächlichen Hornhautlamellen, auf dem Höhepunkt des Prozesses einen Ersatz der oberflächlichen Hornhautlagen durch ein gefässreiches Granulationsgewebe.

Die Veränderungen können derartig tiefgreifend sein, dass es zur Hornhautulceration und zur Einheilung von Irisgewebe in den Hornhautdefekt kommt.

Die Entstehung der menschlichen K.ph. als Kombinationseffekt von photodynamisch wirksamer Substanz und Sonneneinwirkung gewinnt durch diese Versuche an Wahrscheinlichkeit.

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[1] F. D. ENZIE UND G. E. WHITMORE, *J. Am. Vet. Med. Assoc.*, 123 (1953) 237.
[2] K. ULLERICH, K. WULF UND A. WISKEMANN, *Klin. Monatsbl. Augenheilk.*, 131 (1957) 30; *Am. J. Ophthalm.*, 6 (1960) 49.
[3] K. WULF, in GOTTRON-SCHÖNFELD, *Dermatologie und Venerologie*, Kapitel *Lichtdermatosen* Bd. III, 1, Springer, Berlin, 1959.
[4] K. WULF, K. H. MEYER ZUM BÜSCHENFELDE UND K. ULLERICH, *Arch. klin. u. exptl. Dermatol* im Druck.

Experimentelle Untersuchungen über den Einfluss dermatotherapeutischer Faktoren auf die UV-Erythemschwelle

In der vorliegenden Arbeit wurde versucht, einen Einblick in die noch wenig erforschte Beeinflussung der Hautempfindlichkeit gegenüber UV-Licht durch dermatotherapeutische Faktoren zu erhalten. Eine Reihe von Behandlungsmassnahmen für verschiedene Dermatosen, die einerseits äusserlich, anderseits peroral und parenteral zur Anwendung kamen, wurden daher in ihrer Auswirkung auf die UV-Erythemschwelle untersucht. Durch die Bestimmung der UV-Erythemschwelle gelingt es, methodisch die zeitliche Begrenzung der Hautrötung zu beurteilen, die Wucherpfennig[1] 7 Stunden nach einer Bestrahlung mit der Sektorentreppe als noch eben erkennbare Feldumrandung definiert und als Masstab der Lichtempfindlichkeit der Haut ansieht. Ellinger[2] fand mit Hilfe des UV-Erythemschwellenwertes, dass das Lebensalter, Haarfarbe und Geschlecht des Menschen sowie individuelle Schwankungen und Rassenunterschiede und auch jahreszeitliche Schwankungen die Lichtempfindlichkeit beeinflussen können. Hegemann[4] bezeichnet das UV-Erythem als unspezifisches Modell für Hautentzündungen, die, ebenso wie die durch mechanische oder thermische Reize bedingten, allgemeine Gesetzmässigkeiten erkennen lassen. Schönicke[3] fand, dass das sympathisch-parasympathische System je nach Reaktionslage sowohl das UV-Erythem als auch dessen Intensität wesentlich bestimmt. Im Sinne einer Funktionsprüfung der Haut gelingt es demnach, mit der UV-Erythemschwelle die Wirkung verschiedenster Faktoren auf den Organismus sichtbar zu machen.

Bei unseren Untersuchungen wurde die Bestimmung der UV-Erythemschwelle, wie auch von Ellinger[2], Schönicke[3] und Hegemann[4], mit messbar abgestuften UV-Strahlendosen einer Sektorentreppe an der Haut des Rückens vorgenommen. Das Feld, das nach 24 Stunden bei Tageslichtbetrachtung gerade noch eine deutlich sichtbare Rötung mit scharfer Begrenzung erkennen liess, wurde als Erythemschwelle registriert. Um Wärme- oder andere flüchtige Erytheme auszuschalten, wählten wir eine Ablesezeit von 24 Stunden. Nach Böhmer und Liebel[5] sind die Unterschiede zwischen dem 24-Stunden- und 7-Stundenwert als unerheblich zu betrachten. Wir verwendeten für die Bestrahlungstreppe 12 je 1 : 2 cm grosse Felder (E 1 – E 12), die in arithmetischer Reihe, bei einem Hautabstand von 50 cm zur Bestrahlungsquelle, nach 5 Minuten Einbrenndauer fünfzehn Sekunden lang bestrahlt wurden, so dass auf E 1 fünfzehn Sekunden und auf E 12 drei Minuten entfielen. Mit dieser Versuchsanordnung war es möglich, eine genügend genaue Trennung der einzelnen Felder zu erreichen. Zur Bestrahlung diente uns eine Hanauer Quarzlampe S U 500 Wechselstrom, deren Netzspannung mit einem Voltmeter zu kontrollieren und konstant zu halten war.

Zunächst wurde die Bestimmung des Normalwertes der UV-Erythemschwelle für die zu unseren Untersuchungen verwendete Quarzlampe bei 30 als normal und gesund zu betrachtenden Personen durchgeführt. Es ergab sich ein Mittelwert, der in Übereinstimmung mit Voruntersuchern im Feld 5 lag. Darüber hinaus wurde bei jedem Fall der UV-Erythemschwellenwert auch noch vor der Anwendung der zu untersuchenden therapeutischen Massnahmen bestimmt und für die Beurteilung der Unter-

Literatur S. 521

*suchungsergebnisse mit dem Mittelwert in Beziehung gesetzt. Für eine Über- (Unterempfindlichkeit der UV-Erythemschwelle war eine Abweichung von mind*tens 30% zu fordern, die demnach bei einem Unterschied von 3 Feldern gegeben wa

Für unsere Untersuchungen standen uns 73 Fälle zur Verfügung. Bei ihnen wurde folgende Heilmittel angewendet: Cortison (— Prednisolon und Dexamethason) Megaphen (—N-(3'-Dimethylamino-propyl)-3-chlorphenothiacin) und Rimifon (= Iso nikotinsäurehydrazid) bei innerlicher Darreichung; Arsen (als Solarson - Ammoniumsalz der Heptenchlorarsinsäure), Eigenblut und Milch in Form von Injektionen Meladinine (= Ammoidin und Ammidin) zur lokalen Behandlung.

Bei folgenden Hautkrankheiten wurden diese Mittel mit Erfolg angewendet Cortison bei Urticaria, Cheiropompholyx und Alopecia areata; Megaphen bei Ekzem Pruritus, Ulcus cruris und Dermatomykosen; Rimifon bei Lupus vulgaris; Arseninjektionen bei Alopecien und Psoriasis vulgaris; Eigenbluteinspritzungen bei Urticaria; Milchinjektionen bei Lichen ruber planus. Meladinine, das bei Vitiligo unc Alopecia areata gute Heilerfolge zeigte, wurde äusserlich bei Fällen von Urethritis Hautkarzinom und Keloid angewendet, allerdings ohne therapeutischen Zusammenhang mit der vorliegenden Hautkrankheit, da lediglich der Effekt dieses Mittels au nicht krankhaft veränderter Haut des Rückens zu untersuchen war. Die UV-Erythemschwelle wurde daher an der vorher mit Meladinine eingepinselten sowie gleichzeitig zur Kontrolle an einer unbehandelten Stelle der Rückenhaut bestimmt.

Die Untersuchung der UV-Erythemschwelle ergab im einzelnen folgende Mittelwerte: Cortison bei 15 Fällen: Mittelwert E 11. Megaphen bei 15 Fällen: Mittelwer E 3. Rimifon bei 5 Fällen: Mittelwert E 3. Arsen bei 5 Fällen: Mittelwert E 3. Eigenblut bei 5 Fällen: Mittelwert E 9. Milch bei 5 Fällen: Mittelwert E 10. Meladinin bei 23 Fällen: Mittelwert E 2.

Unsere Untersuchungsergebnisse zeigen, dass die angewendeten Mittel in zwe Gruppen einzuteilen sind, die als gemeinsames Merkmal die Beeinflussung d UV-Erythemschwelle im Sinne der Über- oder Unterempfindlichkeit besitzen. Zu einer Überempfindlichkeit der Haut, gemessen an der UV-Erythemschwelle, führte die innerliche Darreichung von Megaphen und Rimifon sowie die Injektion vo Arsen. Innerliche Gaben von Cortison und die Injektion von Eigenblut und Milc bedingten Unterempfindlichkeit. Die lokale Anwendung von Meladinine ergab ein Erhöhung des UV-Erythemschwellenwe··es.

Dieses Verhalten lässt sich bei Arsen mit einer sensibilisierenden Wirkung auf UV Strahlen erklären. Bei Megaphen und Rimifon hängt der Ausfall der Hautreaktio offenbar mit einer Kapillarwirksamkeit dieser Mittel zusammen, die über de Gesamtorganismus zu einem veränderten UV-Erythemschwellenwert führte. Di nachgewiesenen günstigen therapeutischen Erfolge können mit diesen Beobachtunge in Einklang gebracht werden. Bei Meladinine ist das Untersuchungsergebnis ebenfall mit einer durch dieses Mittel bedingten Photosensibilität zu erklären. Die bei Cortiso herabgesetzte Hautempfindlichkeit gegenüber UV-Licht legt die Annahme eine erhöhten Widerstandskraft der Hautreaktion beziehungsweise einer verminderte Entzündungsbereitschaft des Organismus nahe. Die bei Eigenblut und Milch beobachtete gleiche Wirkung auf die UV-Erythemschwelle kann im Sinne des Stressgeschehens (unspezifische Umstimmungsmittel) gedeutet werden und stellt letztlic einen Cortisoneffekt dar.

Zusammenfassend lässt sich festhalten, dass die UV-Erythemschwelle als Haut

funktionsprüfung geeignet erscheint, die Beeinflussung der Hautempfindlichkeit gegenüber UV-Licht durch dermatotherapeutische Faktoren nachzuweisen. Es zeigte sich, dass die Wirkungsweise der angewendeten Heilmittel durch die vorliegenden Untersuchungsergebnisse bestätigt werden kann.

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1 V. WUCHERPFENNIG, *Med. Welt*, (1938) 1841, 1869.
2 F. ELLINGER, *Strahlentherapie*, 20 (1935) 153.
3 K. A. SCHÖNICKE, *Strahlentherapie*, 89 (1953) 469.
4 G. HEGEMANN, *Langenbecks Arch. klin. Chir.*, 262 (1949) 40.
5 L. BÖHMER UND H. LIEBEL, *Strahlentherapie*, 91 (1953) 361.

Seasonal variations in light-sensitivity

The reaction of the skin after exposure to ultraviolet irradiation can be measured partly by the erythema arising from it and partly from skin pigmentation. Earlier investigations by Ellinger[1] have shown that the reaction varies with the seasons if measured in terms of the minimal erythemal dose. Bachem[2] has shown that the time at which erythema and pigmentation appear depends on the wavelength of the ultraviolet rays. Long U.V. light produces erythema and pigmentation almost immediately but the degree and fading-out period vary. In the case of middle and short U.V. light, time reactions are fairly constant so that after 48 h one can count on finding 50% of the erythema arising from short U.V. light and 100% from middle U.V. light. For both wavelengths pigmentation is stated to be 80–100%. Edwards and Duntley[3] have, however, found smaller percentages. The recording of reactions after 48 h will thus represent both erythema and pigment. Hence it will be expedient to introduce the term colour *dose*, which should be distinguished from erythemal *dose*.

This paper, which only deals with the visible skin reactions, is part of a study of reflectance spectrophotometry of the skin colour and reaction upon irradiation with U.V. light. The investigation was carried out from February 1957 to February 1958.

MATERIAL

The material comprised 120 women and 52 men between the ages of 20 and 60, the majority between 25 and 50 years old. They were all members of the hospital staff and only persons in good health and with normal skin were included.

Each person was examined once a month. 80% were examined in all the months of the year, 93 over 11 months, and the remainder over 10 months. For each subject a case-sheet was kept covering the following items: indoor/outdoor work, occupational exposure to U.V. light and ultrasoft X-rays, colour of hair, eyes, and skin, number of freckles, pigmentation, time and place of summer holidays, spare time (indoor/outdoor), and use of sunscreeners.

The material was divided into 3 groups: The first group included 18 persons who are black-haired, dark-skinned, iris usually dark, without freckles, and having strong pigmentation. The second group included 13 persons characterized by sandy hair,

References p. 525

fair skin, and usually light iris, a great number of freckles, and poor pigmentatio The third group comprised 141 persons who do not unambiguously belong in eith the first or the second groups.

METHODS

Fig. 1. Test area: ventral side of the left forearm which was divided into 12 test sec tions, one for each month so that no section was irradiated more than once.

Irradiation procedure: corresponding to the 12 sections, 4 square areas measuring 10 mm by 10 mm at intervals of 10 mm were primarily irradiated. These areas were stamped into a non-lustrous black rubber plate fastened on the skin without its being anaemised. If necessary, a light dry shaving of the test area was performed before irradiation.

U.V. generator: high-pressure mercury arc (Hanau) with relative spectral emission of 25% short U.V., 75% middle U.V. and 100% of long U.V. light.

The U.V. output was calibrated photometrically once a week. Irradiation times were 1, 2, 4, and 8 min so that the shortest irradiation time was always placed distally. If no reaction was observed a symmetrical point of the right forearm was irradiated for 16, 24, and 32 min. Readings were taken after 48 hours and the shortest irradiation time resulting in reaction is referred to as *m*inimal *c*olour *d*ose (m.c.d.).

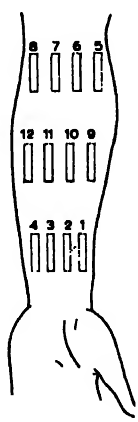

Fig. 1. Test area: Ventral side of the left forarm divided into 12 test sections, one for each month.

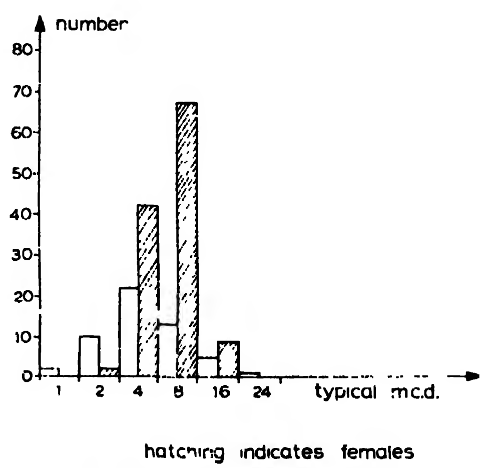

Fig. 2. Abscissa. Typical minimal colou dose (m.c.d.) found for the individual sub ject in at least 6 of the 12 test months Ordinate. Number of persons in each group

RESULTS

The m.c.d. of the subjects is on the whole exactly the same in most months of th year, but it varies from person to person. For the individual subject it has been pos sible to state a typical m.c.d.; by this it is meant that the dose stated was found i at least 6 of the 12 test months. For the female subjects, the typical m.c.d. appear in 8 out of 12 months, for the males only in 6 (Fig. 2). It varies from 1 to 24 min the commonest figures being 4 and 8 min, found in 91% of the females and in 71% c the males. The latter show a greater dispersion. The average m.c.d. for females is 6.6 and for males 6.05 min.

The m.c.d. of sandy-haired subjects does not seem to differ from that of the normal type but the relevant figures are not sufficient for a statistical evaluation. The group of blackhaired subjects has a higher m.c.d., thus 75% normally have an m.c.d. of more than 8 min.

The following factors produced no alteration in the m.c.d.: holiday periods, spare-time occupations, outdoor work, occupational light exposure, and use of sun-screeners (Fig. 3).

Seasonal variations in m.c.d. for men and women appear from Fig. 3. Of statistical significance are the spring maximum, the summer minimum, and in addition an August maximum for the women. In principle the curve is identical for both sexes;

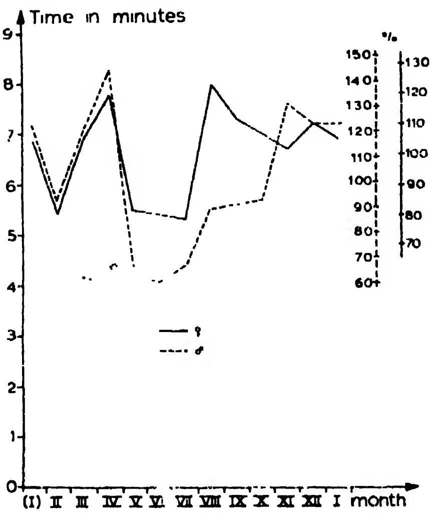

Fig. 3. Seasonal variations in m c d. for all men and women during a year. Left ordinate m.c.d. in minutes, to the right in percent.

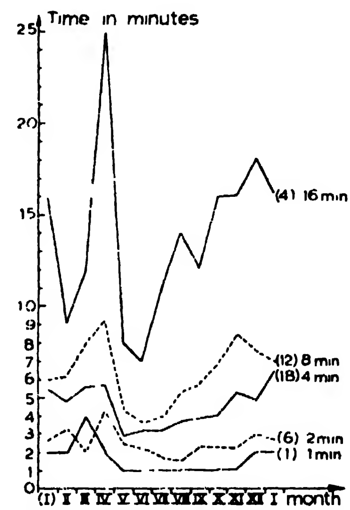

Fig. 4 Male in general: Seasonal variations in m.c.d., grouped in typical m c.d. Figures in parenthesis refer to the number of persons.

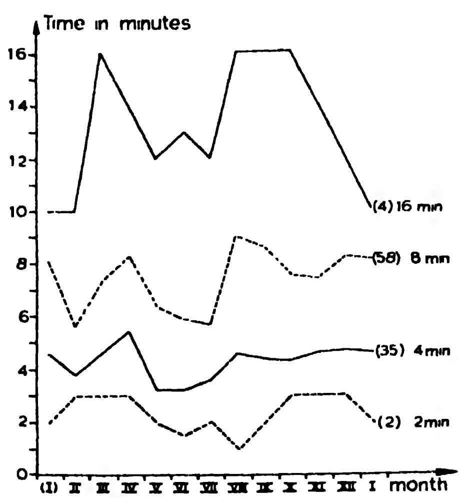

Fig. 5. Female in general: Seasonal variations in m.c.d., grouped in typical m.c.d. Figures in parenthesis refer to the number of persons.

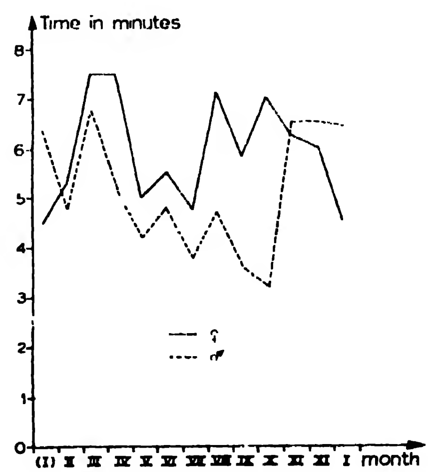

Fig. 6. Seasonal variations in m.c.d. in the group of sandy persons. Red haired and fair skinned persons.

References p. 525

however, the men show greater variation over the year than the women. In the wome we find variations ranging from 124% to 77%, in the men from 145% to 60%. I further appears that women are 10% less light-sensitive than men.

The variations observed over the year are identical in all groups irrespective o their typical m.c.d. This appears from Fig. 4, which shows seasonal variations withi individual groups having the same m.c.d. Fig. 5 represents the same division in th case of the female subjects; the characteristic August maximum is here observable ir all groups. To account for the cause of the August maximum demonstrated in female it was considered important to examine the seasonal variations found in the 2 group representing the extremes of the material, *viz.* the sandy-haired subjects with poo pigmentation and the black-haired subjects with strong pigmentation.

Fig. 7. Seasonal variations in m c.d. in the group of black-haired and dark-skinned persons

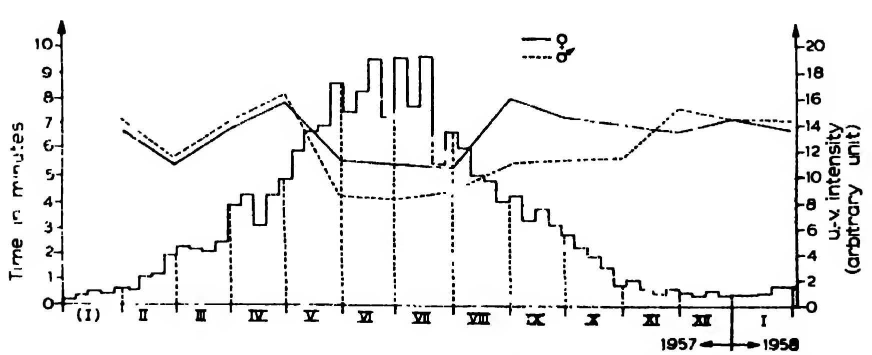

Fig. 8. Seasonal variations in m.c.d. The ultraviolet light intensity found over the city of Copen hagen, recorded for each week in arbitrary units.

Fig. 6 shows the group of sandy persons. Seasonal m.c.d. variations are similar tc those in the general group, which means that the women keep up their August maximum. The decrease observable simultaneously in the men is not significant, so they may be said to follow the variations of the general group.

Fig. 7 represents the group of black-haired persons. To a great extent the curve is

similar as regards both males and females and on the whole it corresponds with the curve for females. This can only mean that it is the increased pigment content of the skin which causes the female August maximum and that the high m.e.d. results from the screening effect of the pigment.

It should be emphasized that in a sense the material is selected, the hospital nurses wearing short-sleeved uniforms and the men wearing jackets; besides, the men's hairier skin may impede the effect of sunlight.

Fig. 8 again gives the seasonal variations in terms of m.e.d., but this time considered in relation to the ultraviolet light intensity found over Copenhagen. These results were kindly placed at our disposal by Dr. Ebbesen. The light intensity was recorded for each week in arbitrary units. This curve does not differ essentially from light intensities measured in other years and must be considered representative as regards the norm. It may be added that it is usually possible to stay outdoors lightly clad from April/May to September/October, more precisely perhaps from May to September.

It will be noted that simultaneously with the heavy increase in light intensity during May there sets in a decrease in m.e.d. which remains on the same level in spite of greater light intensity during June and July. If the decrease in light intensity during August were of any importance it would manifest itself in both sexes. However, an increase is only seen in the women, a fact ascribable to the sun-screening effect of their pigment.

Towards the close of the year we again observe the same values for men and women with a minimum during February corresponding to the decrease in skin pigmentation. This agrees with the results stated by Edwards and Duntley[3], who have demonstrated that after one month the amount of newly formed melanin begins to decline, and after 9 to 10 months the melanin content of the skin is almost back to normal. The maximum demonstrated for April is therefore mainly caused by the erythemal component of the m.e.d.

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J. V. Christiansen
H. Brodthagen

[1] F. Ellinger, *Medical Radiation Biology*, Charles C. Thomas, Springfield, Ill., 1957.
[2] A. Bachem, *Am. J. Phys. Med.*, 35 (1956) 177.
[3] E. A. Edwards and S. Q. Duntley, *Am. J. Anat.*, 65 (1939) 1.

Introductory remarks for the three papers on the effects of therma radiation on skin

Mr. Chairman – Ladies and Gentlemen:

The three papers I am privileged to deliver deal with effects of intense radiation o skin for relatively short periods. The effects dealt with are those caused only by th raising of the temperatures of skin because of absorption of energy and its conversio to heat. The effects are the familiar burns, blisters, etc., and resemble those caused b contact with hot objects or liquids and flame. The subject matter should be of intere to all research workers on skin, not only those of us concerned with burns, first - because absorption of energy by skin raises its temperature, changing the condition (the experiment, and second — the degradation or burning of tissue by the elevate temperatures may be an unwanted occurrence confusing the results of an experimen My first paper will deal with the physical properties of skin which are important i knowing what temperatures to expect – my second paper deals with temperatu histories which are associated with burns, and the third paper on the effects of reactic of skin as part of the body system as they might affect and modify the flow of he and local temperatures.

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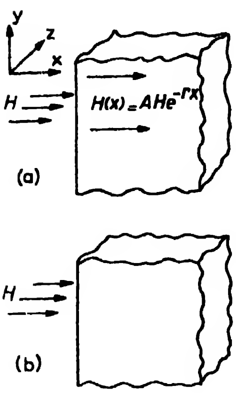
The physical properties of skin involved in thermal radiation burı

The temperature history of the skin during and immediately following exposure ı intense thermal radiation is an important factor in determining the nature of burı caused by the absorption of radiant energy by skin. While there are differences in tl properties of skin among individual persons and among the areas of one individual skin, it is desirable to have a set of constants with a probable range of values. Tl constants are to be employed in the study of radiant energy heating to exami the dependence of temperature on various factors and to serve as a reference f specific problems.

The mathematical model which represents skin is that of a diathermanous solid which the absorption and scattering of thermal radiation vary with wave length aı depth, and the other thermal properties also vary with depth. However, the analy ical solution for heat flow in such a model is complex and it is difficult to devise e periments for measuring the constants precisely. As a first approximation we c employ a greatly simplified model which assumes the skin to be homogeneous, aı in which the energy is absorbed exponentially with depth and for which the prop ties are constant. The analytical expressions for the temperature histories in dep

and on the surface for this simple diathermanous model are given in Fig. 1. The equations are for a monochromatic source; for a heterochromatic source it would be



$$\theta(x,t) = \frac{AH}{k}\left\{\frac{2u}{r\sqrt{\pi}}e^{-v^2} - x \operatorname{erfc} v + \frac{e^{-u^2}}{2r}\left[e^{-rx}\operatorname{erfc}(u-v) + e^{rx}\operatorname{erfc}(u+v)\right] - \frac{e^{-rx}}{r}\right\}$$

$$\theta(0,t) = \frac{2AHt^{1/2}}{\sqrt{\pi k\varrho c}}\left[1 + \frac{\sqrt{\pi}}{2ru}e^{u^2}\operatorname{erfc} u - \frac{1}{r}\right]$$

$$\theta(x,t) = \frac{2AHt^{1/2}}{\sqrt{\pi k\varrho c}}\left[e^{-v^2} - \sqrt{\pi}\, v \operatorname{erfc} v\right]$$

$$\theta(0,t) = \frac{2AHt^{1/2}}{\sqrt{\pi k\varrho c}}$$

θ Temperature rise (°C)
A Radiant absorptance
H Irradiance (cal/cm²/sec)
t Time of exposure (sec)
h Thermal diffusivity $= k/\varrho c$
ϱ Density (gm/cm³)
k Thermal conductivity (cal/sec cm °C)
c Specific heat (cal/g °C)
r Extinction coefficient (cm⁻¹)
x Distance from surface (cm)
u $= r\sqrt{ht}$
v $= x/2\sqrt{ht}$

$$\operatorname{erfc} \xi = 1 - 2/\sqrt{\pi}\int_0^{\xi} e^{-\eta^2}\,d\eta$$

Fig. 1. Temperature rise due to radiant heating in (*a*) a diathermanous and (*b*) an opaque, semi-infinite solid.

necessary to sum the temperature rises caused by the various wave length regions for which the skin had significantly different properties.

The absorptance of skin is a function of wavelength of the impinging radiation. In general, in the ultraviolet region and in the infrared for wavelengths longer than 1.5 microns, the energy is virtually all absorbed at the surface, while in the visible and near infrared regions selective penetration and scattering takes place and significant amounts of energy are scattered back through the surface and do not contribute to heating of the skin. The total amount of energy absorbed by the skin for a heterochromatic source may be calculated, if the spectral absorptance is known, by summing the energy absorbed over the spectral range of the source.

The opaque-solid model also shown in Fig. 1 is simpler to deal with and allows us to examine the thermal properties of skin without the complexities which must be considered when the radiant energy also enters the skin. Opaqueness can be achieved readily by painting the skin and various techniques are available for measuring surface temperatures. The principal parameter determining the surface temperature of an opaque solid is the $k\varrho c$ product.

The principle physical properties which are important in analytical studies of heat flow in skin are given in the table with a suggested reference value and the probable range of these values.

The reference $k\varrho c$ product is based on that determined at this laboratory by meas-

TABLE I

TABLE OF SKIN PROPERTIES

	Reference	Minimum	Maximum
$k\varrho c$ product (cgs units $\times 10^{-4}$)	8.6	4	17
$k/\varrho c$ (cgs units $\times 10^{-4}$)	15.1	6.1	26
Absorptance for 3000°K blackbody spectrum	0.72	0.65	0.79
for carbon-arc spectrum	0.72	0.66	0.77
for solar spectrum	0.63	0.47	0.85
Extinction coefficient (cm^{-1})	30	10	∞

uring temperatures on the skin of the inner forearm. The opaque solid model was employed with temperature rises obtained in exposures of one second. This value would be appropriate for exposure situations in which reactions to elevated temperatures would not be involved and in which surface temperatures would not be unduly affected by the thermal properties at greater depths in the skin. The maximum value was found for the skin of the back of the hand while the minimum value would represent surface temperatures in the palm of a work-worn hand.

The values of the thermal diffusivity of skin were computed employing the $k\varrho c$ product and a volumetric specific heat of 0.81 cal/cc.

The reference absorptance was calculated for the various spectra employing the average of the diffuse spectral absorptance as measured in our laboratory on four Caucasian and two medium Negro subjects. The maximum absorptances are those for a dark Negro as calculated from an absorption spectrum measured by Dr. H. Kuppenheim and the minimum values are those calculated similarly for a light Caucasian subject.

The reference extinction coefficient suggested is that value which would result in the measured surface temperature of a lightly tanned Caucasian skin after one second exposure to the radiation spectrum of the high-intensity carbon arc. The maximum value, that for an opaque solid, will obtain for ultraviolet radiation and also infrared energy for wave lengths longer than 1.5 microns. The minimum value is that measured for excised skin by Dr. J. D. Hardy for radiation having a wavelength of 1.23 microns.

In using these constants it must be remembered that factors such as blood flow and the systemic reaction of skin to levated temperatures change the heat flow in skin so that corrections must be made to expressions for temperature which are based on an inert-homogeneous-solid model.

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The temperatures associated with thermal radiation skin burns

Knowledge of the temperature histories which will cause burns in blackened human skin is important as a special case of the general problem of temperatures and heat flow in the study of burns caused by intense thermal radiation.

Heat flow is much simpler in blackened skin because of the absence of penetrating radiation; the exposure conditions are more reproducible, and the situation is more amenable to mathematical representation.

The temperature histories of the surface of skin associated with threshold burns can be employed to obtain a first approximation of temperatures in depth which cause the tissue degradation in burned skin. The surface-temperature histories of blackened skin, while for a relatively simple situation, can be used by comparative methods to estimate the burn severity to be expected in other more complex situations, for which the temperatures are obtained by experiment or calculations.

While it is feasible to produce burns on human skin it is more practical to use animals to develop the experimental techniques for studying the general phenomenology of burns. In this study threshold burns were produced on blackened, anesthetized rats with exposures to radiant energy ranging in duration from 0.5 to 100 sec. The energies per unit area to cause such burns are given in Table I, as are the temperature-rise maxima measured during the series of burning episodes.

TABLE I

THE RADIANT EXPOSURES REQUIRED TO PRODUCE A THRESHOLD BURN TO BLACKENED HUMAN SKIN

Based on burns to blackened rats for exposures of 0.5 to 100 sec

Exposure time sec	Radiant exposure: Rats (experimental) cal/cm^2	Human skin (calculated) (a) cal/cm^2	Maximum temperature rise per unit radiant exposure: Rat °C/cal/cm^2	Human °C/cal/cm^2	Maximum temperature rise for a threshold burn °C
0.5	0.8	1.0	70	56	56
1.0	1.2	1.4	46	38	53
2	1.5	1.7	30	26	44
5	1.7	1.7	17	17	29
10	2.2	2.2	12	12	26
20	2.8	2.5	8.0	9	22
50	3.7	3.1	5.1	6	19
100	4.4	3.0	3.4	5.0	15

(a) Calculated on the basis of achieving the same maximum temperature.

To translate these data into the equivalent radiant exposures which would cause threshold burns to human skin in identical situations is not straightforward. Rat skin, physically and biologically, is significantly different from human skin. While differences in optical properties have been eliminated by the opaque blackening on the skin it can be demonstrated that, for equal input, differences in thermal properties result in significantly different surface temperatures. The maximum temperature

rises for unit exposure for human skin, also given in the table, were obtained for small thermal inputs and indicate the differences between the two skins.

The initial surface temperature of the rat skin just before exposure was 31 ± 1° and, since that of human skin is often the same, no correction based on initial surface temperature is here attempted. It was assumed that a threshold burn would be produced on human skin if its temperature were raised to the same maximum temperature as that which produced a threshold burn in rat skin in the same time. The radiant exposures which would cause burns to human skin on the basis of this assumption are presented in Table I.

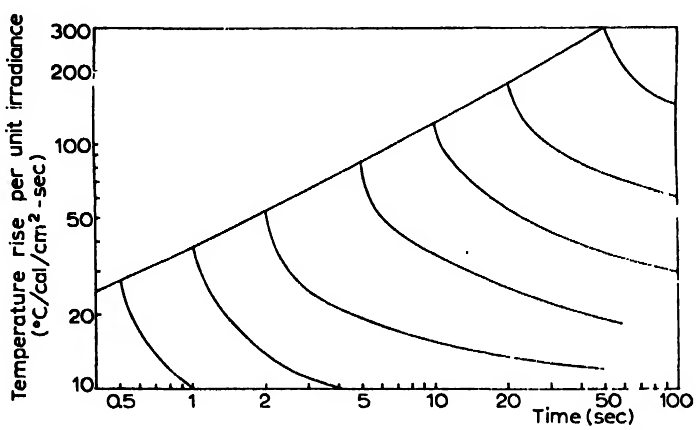

Fig. 1. The temperature history of the surface of the blackened skin of the inner forearm for temperatures below the pain threshold.

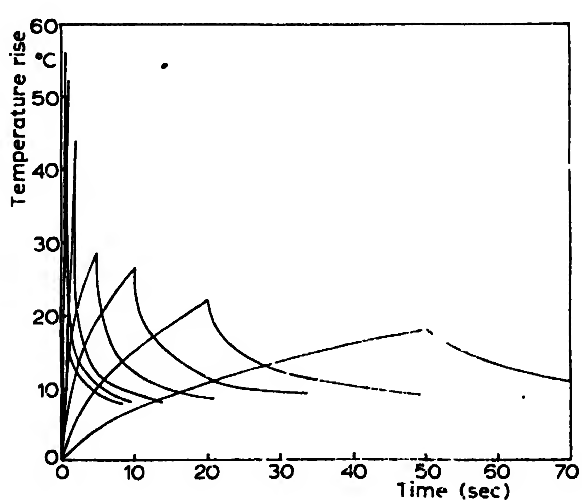

Fig. 2. Temperature history of the surface of blackened skin associated with threshold burns.

The measured temperature histories of the surface of blackened human skin are given in Fig. 1. The histories are the average of a series of measurements for temperature rises less than pain on the inner forearms of 6 males, representing areas of the body which are normally covered.

The temperature histories corresponding to threshold burns are obtained by using the appropriate energy inputs and are shown in Fig. 2. The maximum temperature rises causing burns range from 56° for a 0.5 sec exposure to 15° for a 100 sec exposure. Assuming an initial surface temperature of 31°, the corresponding maximum temperatures would range from 87° to 46°. The temperature histories depict the shorter durations of the higher temperatures and indicate the operation of rate processes for tissue degradation.

The temperature histories given are those for the surface of skin; temperature histories in depth may be derived from the surface temperatures by the application of heat flow theory, but are subject to variation due to blood flow and inhomogeneity in skin properties with depth.

The temperature histories given are subject to probable errors as great as 10% in the blackened skin temperature measurements and also in determining the radiant exposure to cause the threshold burn.

The data presented represent a relatively important situation. The necessity for carefully measuring the temperatures of the skin when studying burns is indicated by the range of temperature histories and the effect that skin condition and its initial temperature would have on the resulting lesion.

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Systemic reaction of skin to prolonged local heating

Study of burns to skin caused by intense thermal radiation requires knowledge of the temperature histories of the skin during and immediately after exposure to intense thermal radiation. In order to predict the temperatures associated with long-duration exposures on the basis of theoretical considerations, it may be necessary to make allowance for the effect of blood flow, spatial variation in the thermal properties of skin, changes in evaporation rates, and other unknown factors.

The study reported herein includes irradiation periods up to 50 sec and temperature measurements of blackened skin for as long as 100 sec after insult.

For several years the Naval Material Laboratory has been investigating the phenomenology of burns caused by intense thermal radiation and methods of protecting personnel against such burns. As part of this study an inert skin simulant with imbedded thermocouple was developed. White rat skin has also been employed as a substitute for human skin. The temperatures of human skin in sub-critical situations have been of direct value in correlating rat and simulant data with the corresponding human situation.

The mathematical model representing heat flow in skin is that of a diathermanous solid wherein the absorption and scattering in depth of thermal radiation is wavelength dependent, the properties vary with depth and heat sources and sinks may be affected by changes in the temperature of the medium. This report neglects the optical

properties and concerns itself only with thermal properties and their variation.

The thermal properties of skin have been investigated by many laboratories, apparent variations of these properties with time during exposure to radiant energy have been reported. The conduction parameter of human skin, the $k\varrho c$ product, has been found to increase as the exposure time is increased. The apparent change in $k\varrho c$ with time of exposure may arise from an increase in conductivity with depth or the action of other heat transport mechanisms. The fact that skin is part of a living organism with blood flowing near the surface makes it capable of changing the heat flow pattern by reacting to elevated temperatures during the episode in question.

As a first approximation the effects of reradiation losses, inhomogeneities in skin, and blood flow may be neglected for times less than one second and $k\varrho c$ may be obtained directly from measured temperature rises. The effect of reactions within the system will be minimal and, if the area of exposure is sufficiently large, the effect of tangential blood flow will also be small. The temperatures of skin, in the first instants of an exposure, will be those for an inert homogeneous solid with the properties of the skin near the surface. As the exposure continues, the $k\varrho c$ will apparently increase as the blood transports energy, and will be further modified if the deeper tissues have properties different from those near the surface.

Experimental temperature histories were obtained by exposing the inner surface of the subject's blackened forearm to the radiant output of a 1000 W tungsten filament lamp as shown in Fig. 1. To obtain temperatures not influenced by blood flow, exposures were made with a pressure cuff at 180 mm of mercury on the upper arm; for temperatures affected by flow, the blood flow was occluded for from 2 to 3 min and exposures were initiated just after release of the pressure cuff. The period of high blood flow normally continues for about 20 sec unless sustained by elevated temperatures. Series of exposures on six individuals were made over a period of several hours. Sets of such exposures were made over a period of 10 weeks, usually with several days between each series. The volunteer subjects were males drawn from the Laboratory's staff, ranging in age from 30 to 45 years.

Fig. 2 presents the average of the temperatures measured, normalized to that for unity irradiance. As evident from the appearance of relaxation temperatures in the

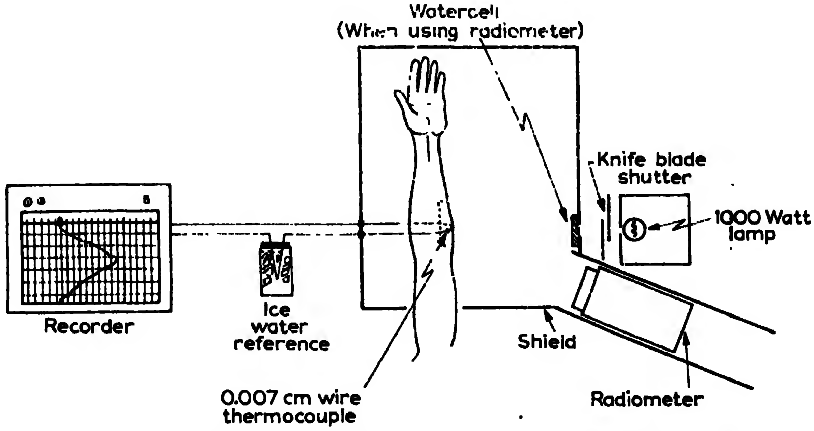

Fig. 1. Experimental apparatus employed in measuring skin temperature.

figure, exposures were variously terminated at 1, 2, 5, 10, 20, and 50 sec. The temperatures for maximum blood flow were found to be nearly identical to those with normal or unaltered blood flow shown in the previous paper. The temperatures for occluded blood flow were about 15% higher than those for full flow independent of time or

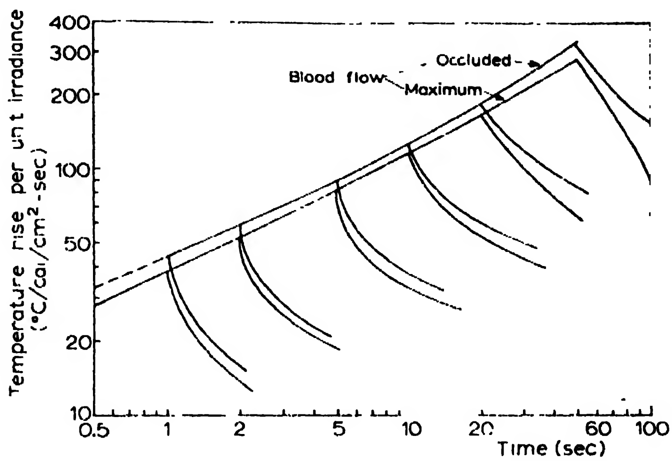

Fig. 2. Temperature histories for blackened human skin for maximum blood flow and blood flow occluded.

exposure duration. There was, however, a tendency for larger variations from the norm after the cessation of the longer exposures. The temperatures with blood flow occluded were higher for some individuals for the longer exposures than would be expected on the assumption of a homogeneous solid. A lower conductivity for the deeper tissues is indicated. The magnitude of this effect is not as great as the differences found for the temperature histories among the six individuals.

In general one can say, from this limited experiment, that for skin temperatures generated by intense thermal radiation, measurable differences are caused by blood flow and therefore reactions of the body system, by changing the flow, would cause variations which could be significant.

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Zur Wirksamkeit des in der Haut durch ultraviolette Strahlen erzeugten Vitamins D*

Bei einem Kollektiv lebender Albinoratten im Alter von 21–24 Tagen wurden unter Abdeckung des übrigen Körpers[1,2] die Schwänze 30 Min. lang mit dem Gesamtspektrum eines Hg-Hochdruckbrenners vom Typ S 300 im Abstand von 50 cm einseitig dorsal bestrahlt. 22 Tiere wurden sofort nach UV-Exposition getötet, die Schwänze abgetrennt, erschöpfend extrahiert und nach Bestimmung des Vitamin-D-Gehaltes dieser Extrakte ermittelt, wieviel Vitamin D pro Rattenschwanz und damit pro Ratte durch die UV-Bestrahlung erzeugt worden war. Wir fanden 0.41 μg Vitamin D pro Rattenschwanz. Die gleiche Anzahl Tiere blieb am Leben. Sie dienten zu einem Vergleich der antirachitischen Wirksamkeit der Bestrahlung mit der Wirkung peroral bezw. intramuskulär verabreichten Vitamins D. Insgesamt wurden dazu 4 Gruppen zu je 22 Jungratten gebildet. Die Tiere der einzelnen Gruppen erhielten einmalig
(*a*) lediglich 0.1 ml indifferentes Pflanzenöl p.o.,
(*b*) 0.4 μg Vitamin D_3 in 0.1 ml indifferentem Pflanzenöl p.o.,
(*c*) 0.4 μg Vitamin D_3 in 0.1 ml indifferentem Pflanzenöl i.m.
(*d*) 30 Minuten Schwanzbestrahlung
und vom Behandlungstage an eine rachitogene Diät (McCollum Nr. 3143). Es wurde nun die Entwicklung der Rachitis unter den einzelnen Behandlungsarten verfolgt. Dazu wurden alle 3½ Tage Röntgenaufnahmen der Tibiaepidiaphysenlinie des rechten Hinterbeines der Ratten angefertigt und mittels ihrer wurde der Grad der Rachitis anhand einer Vergleichsskala[3] festgelegt. Das Ergebnis zeigt die Abbildung.

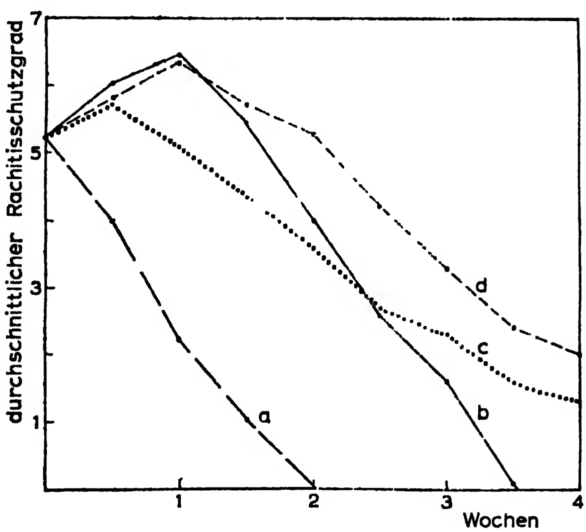

Abb. 1. Rachitisentwicklung in den Gruppen a–d. Ordinate: Durchschnittlicher Rachitisschutzgrad pro Gruppe, Abszisse: Zeit in Wochen. Rachitisschutzgrad O entspricht einer starken Rachitis.

* Herrn Prof. Dr. P. Wels in Ehrerbietung zum 70. Geburtstag.

Wir möchten der Abbildung folgendes Ergebnis entnehmen:

1. Die bestrahlten Tiere sind in mindestens gleichem Masse gegen Rachitis geschützt wie durch intramuskuläre Applikation. Wenn man voraussetzen darf, dass das intramuskulär verabreichte Vitamin D in quantitativ gleicher Weise vom Organismus verwertet wird wie durch Bestrahlung einverleibtes, darf man schliessen, dass das durch Bestrahlung in der Haut gebildete Vitamin D quantitativ oder annähernd quantitativ in den Körper übergeht. Vitamin D-Verlust durch sich abschilfernde Hornschichten kann in nur unwesentlichem Masse vorliegen.

Die Vitamin D-Bildung findet also vornehmlich oder ausschliesslich in Hautschichten statt, die am Stoffwechsel teilnehmen.

2. Die genauere Betrachtung des Verlaufs der Wirkungskurven zeigt, dass der durchschnittliche Rachitisschutz bei den bestrahlten Tieren wie nach oraler Applikation schnell über die für dieses Alter geltende Norm ansteigt, dann aber wesentlich länger anhält. Bezüglich der Wirkungsdauer scheint die Bestrahlung der intramuskulären Applikation zu gleichen. Insgesamt weist also die Wirkung des Vitamins D der Haut auf Grund seines schnellen aber gleichzeitig anhaltenden Effektes in therapeutisch günstiger Weise die Vorzüge der oralen und intramuskulären Applikation auf.

Aus dem protrahierten Wirkungsverlauf ist zu schliessen, dass das durch ultraviolette Strahlen in der Haut gebildete Vitamin D nach Art eines Depots wirkt. Der schnelle Wirkungseintritt dürfte darin begründet liegen, dass es dem Organismus auf breiter Fläche angeboten wird.

Der im Vergleich zur intramuskulären Applikation grössere antirachitische Gesamteffekt der Bestrahlung könnte dadurch bedingt sein, dass in der Haut im vorliegenden Fall mehr als 0.41 μg Vitamin D pro Tier gebildet wurden, oder dadurch, dass das Vitamin D in der Haut in für den Organismus sehr ökonomisch verwertbarer Form vorliegt. Mit anderen Bestrahlungszeiten (45–60 Min.) und D-Dosen (0.50–0.60 μg) wurden entsprechende Ergebnisse erhalten.

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[1] G. PFENNIGSDORF UND W. PONSOLD, *Proc. 1st Intern. Photobiol. Congr.*, Amsterdam, 1954, p. 232.

[2] H. BEKEMEIER, G. PFENNIGSDORF UND W. PONSOLD, *Atti 2 Congr. Intern. Fotobiol.*, *Turin*, 1957, p. 255.

[3] H. BEKEMEIER, *Acta Biol. et Med. Ger.*, 4 (1960) 283.

Session 5

PHOTOBIOCHEMISTRY

Secretary: PER ROSENKILDE, Copenhagen (Denmark)

Strahlenempfindlichkeit und Mechanismus der Enzymsynthese in Bakterien

Die vorliegende Arbeit befasst sich mit der Einwirkung von Röntgenstrahlen und ultraviolettem Licht auf die Induktion des Enzymproteins der Lysin-Dekarboxylase in *Bacterium cadaveris*. Die Bedingungen für die Induktion wurden von Gale und Epps[1] und von Mandelstam[2] untersucht. Da der zuletzt erwähnte Autor gezeigt hat, dass die Enzymaktivität der Menge des Enzymproteins proportional ist, kann die Enzymsynthese in den lebenden Zellen manometrisch durch die Dekarboxylierung von L-Lysin verfolgt werden. Nach Zusatz von Lysin zu den ohne Lysin kultivierten Zellen steigt nach einer kurzen Latenzzeit die Enzymaktivität linear mit der Zeit an. Die Anstiegsgeschwindigkeit — Induktionsrate — schwankt von Versuch zu Versuch um nicht mehr als 10%. Während der Induktion ist die Zahl der lebenden Bakterien konstant. Das Frischgewicht der Bakterien nimmt während dieser Periode um nicht mehr als einige Prozent zu.

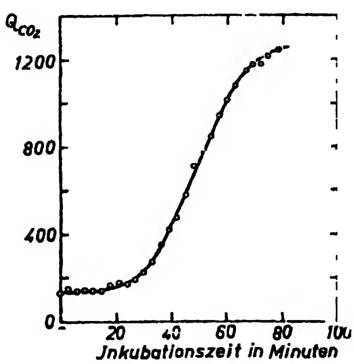

Abb. 1. Enzymaktivität in Abhängigkeit von der Zeit während des Induktionsvorganges. Die Enzymaktivität wurde bei 30° manometrisch bestimmt. Das 15 ml fassende Reaktionsgefäss enthielt 200 μMol DL-Lysin, 30 mg Glukose, 1.5 mg DIFCO-Hefe-Extrakt und etwa 1 mg Bakterien (Trockengewicht) in 3 ml $M/20$ Phthalat-Puffer pH 5.6. 5 Vol. % CO_2 + 95 Vol. % N_2 Atmosphäre. Die Aktivität Q_{CO_2} ist die von 1 mg Bakterien (Trockengewicht) pro Stunde aus Lysin entwickelte Menge CO_2 in mm³.

Durch Röntgenbestrahlung wird die Induktionsrate verringert. Die Enzymaktivität und damit die Menge des Enzymproteins nimmt ebenfalls linear mit der Inkubationszeit zu. Die Dosiseffekt-Kurve lässt sich durch eine Exponentialfunktion mit einer Inaktivierungsdosis von 50 Kr annähern. Da die Inaktivierungsdosis für das bereits vorgebildete Enzym 2 Grössenordnungen höher liegt, kann es sich hier nicht um eine Röntgenstrahleneinwirkung auf das Enzymmolekül selbst handeln, sondern um die Einwirkung auf den Neubildungsmechanismus. Auf Grund der Lea'schen Theorie[3] und unter Berücksichtigung der sicher vorhandenen "indirekten Strahlenwirkung"[4-6] ergibt sich ein strahlenempfindliches Volumen, das einem Molekulargewicht von 10–100 Millionen entspricht. Es liegt nahe, dieses strahlenempfindliche Volumen mit

Literatur S. 541

dem Volumen der synthetisierenden Einheit zu identifizieren und von dem "Molekulargewicht" des "Synthesezentrums" zu sprechen.

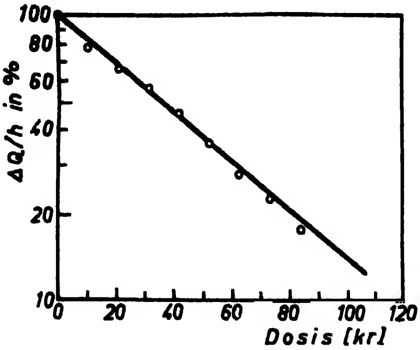

Abb. 2. Beeinflussung der Induktionsrate durch Röntgenstrahlen. 0.1 ml einer gewaschenen Bakteriensuspension mit 50 mg Trockengewicht pro ml $M/100$ Phosphatpuffer pH 6.8 + 0.5% NaCl wurden mit einer Berylliumfenster-Röhre bei 100 kV, 23 mA und 0.5 mm Al-Filter bestrahlt.

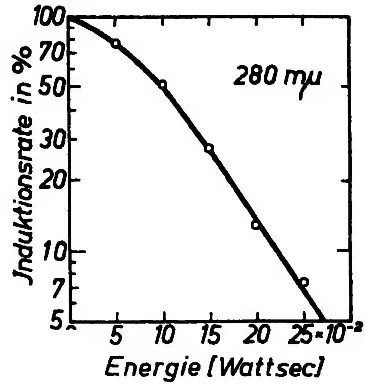

Abb. 3. Dosiseffekt-Kurve für die Induktionsrate nach UV-Bestrahlung mit Licht der Wellenlänge 280 mμ.

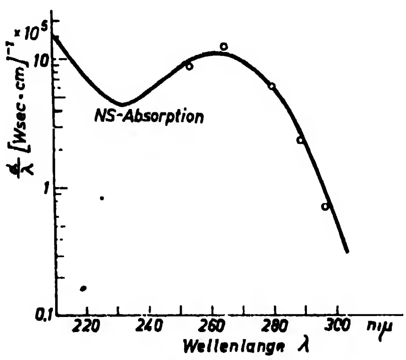

Abb. 4. Lysin-Dekarboxylase. Wirkungsspektrum für die Induktionsrate. Die Punkte stellen die mit den Wellenlängen 254 mμ, 265 mμ, 280 mμ, 289 mμ und 297 mμ gewonnenen v-Werte dividiert durch die Wellenlänge λ dar.

Diese Hypothese wird durch Versuchsergebnisse mit Inhibitoren, wie Natriumazid und Dinitrophenol, unterstützt.

Durch Bestrahlung mit ultraviolettem Licht lässt sich die Induktion hemmen. Die Bestrahlung fand mit einem leistungsstarken Monochromator[7] statt. Die auf die Bakteriensuspension auffallende Energie wurde mit einem Bolometer absolut gemessen. 4.7 ml einer Suspension mit 1 mg Bakterienfeuchtgewicht/ml in Phosphatpuffer wurden in Plexiglasschälchen mit 11 cm² Oberfläche bestrahlt.

Die Dosiseffekt-Kurven sind, wie Abb. 3 zeigt, nicht exponentiell, sondern zeigen das Verhalten der Mehrtrefferfunktionen. Ein Mass für die Strahlenempfindlichkeit wurde mit Hilfe der Formel

$$v = \frac{R + 1}{e^{\alpha D} + R}$$

die auf Grund treffertheoretischer und reaktionskinetischer Überlegungen[8] abgeleitet

wurde, gewonnen. In dieser Formel bedeuten ν die relative Induktionsrate, D die eingestrahlte Energie in Wattsec, $\varkappa$ der Koeffizient, der die Strahlenempfindlichkeit angibt und R eine Konstante. Die ausgezogene Kurve in Abb. 3 stellt die obige Funktion mit $\varkappa = 17.6$ [Wsec]$^{-1}$ und $R = 4.1$ dar.

Die Wellenlängenabhängigkeit der Strahlenempfindlichkeit ist in Abb. 4 eingetragen. Das Wirkungsspektrum hat ein Maximum bei etwa 260 mμ. Der Vergleich mit dem Absorptionsspektrum der Nukleinsäure legt den Schluss nahe, dass es sich beim Wirkungsspektrum um ein Nukleinsäurespektrum handelt. Ein gleiches Wirkungsspektrum wurde bereits von Swenson[9] für das Galaktozymase-System in Hefezellen gefunden.

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1 E. F. Gale and H. M. R. Epps, *Biochem. J.*, 38 (1944) 232.
2 J. Mandelstam, *J. Gen. Microbiol.*, 11 (1954) 426.
3 D. E. Lea, *Actions of Radiations on Living Cells*, University Press, Cambridge, 1946.
4 W. M. Dale, in A. Hollaender (Editor), *Radiation Biology*, I, McGraw-Hill, New York, 1954, p. 255.
5 B. Rajewsky, *Brit. J. Radiol.*, 25 (1952) 550.
6 F. Hutchinson, *Radiation Research*, 7 (1957) 473.
7 H. Bücker, *Strahlentherapie*, 94 (1954) 292.
8 H. Pauly und B. Rajewsky, *Strahlentherapie*, 99 (1956) 383.
9 P. A. Swenson, *Proc. Natl. Acad. Sci., U.S.*, 36 (1950) 699

Ultraviolet inactivation of ribonucleic acid

Action spectra have shown that reproductive death in a wide variety of cells is a consequence of the absorption of U.V. light by nucleic acid. In the grasshopper neuroblast, microbeam experiments have shown that the nucleic acid involved is the nucleolar ribonucleic acid (RNA)[1]. Less direct evidence indicates that nucleolar RNA is also the site of U.V. action in *Neurospora*[2]. Moreover, the surprisingly small variation in U.V. dose as compared with the corresponding X-ray dose required to inactivate cells of very different sizes is consistent with the hypothesis that unlike X-rays, which act on chromosomes[3], U.V. acts on nucleolar, or at least nuclear, RNA. For these reasons it would seem that the RNA-containing viruses and the infectious RNA obtained from them provide good models for investigating the action of U.V. on living cells.

The nucleic acids derived from poliovirus and tobacco mosaic virus are very similar in molecular weight and base composition; but they are very different biologically. (It has not been possible, for example, despite considerable effort, to demonstrate any effect of TMV RNA on mammalian cells that are readily infected by PV RNA.) This biological difference is reflected in at least one large physical difference – PV RNA is inactivated by heat at 70° almost ten times faster than TMV RNA[4]. Apparently, however, the radiation sensitivity is determined predominantly by the molecular weight and gross structure so that the sensitivity to both U.V.[5] and X-rays[4] is quite similar for the two molecules.

References p. 542

The quantum efficiency for the U.V. inactivation of the two nucleic acids is about 10^{-3} (ref.[5]). This is about an order of magnitude smaller than the quantum efficiency for the photolysis of the pyrimidines within RNA as observed by changes in the absorption spectrum[6]. It seems, therefore, that the addition of water across the 5,6 double bond of the pyrimidines—the reaction involved in photolysis—does not in general lead to the destruction of the biological activity of the RNA. This conclusion receives some further support from the failure to demonstrate any recovery of activity upon heating U.V.-irradiated RNA for periods during which some reversal of photolysis was expected[4].

Finally, it is noteworthy that the presence of the protein in the intact virus can change the U.V. sensitivity of the RNA. This effect is most marked in the case of the common strain of TMV which is about an order of magnitude less sensitive to U.V. than its RNA[7-9]. This increased resistance of the RNA coupled with the twenty to one ratio by weight of protein to RNA leads to an action spectrum very different from the absorption spectrum of RNA[10]. Despite this dramatic example of protein — RNA interaction, it seems quite reasonable to assume a quantum efficiency of about 10^{-3} for the inactivation of RNA within cells. If this is the case, then the relatively small spread in U.V. doses required to inactivate a wide variety of cells, even when account is taken of the differences in the amount of nuclear shielding provided by the cytoplasm, presumably reflects a correspondingly small variation in the total quantity of essential nuclear RNA.

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1 M. E. GAULDEN AND R. P. PERRY, *Proc. Natl. Acad. Sci. U.S.*, 44 (1958) 553.
2 A. NORMAN, *Exptl. Cell Research*, 2 (1951) 454.
3 H. H. LEE AND T. T. PUCK, *Radiation Research*, 12 (1960) 340.
4 A. NORMAN, Unpublished.
5 A. NORMAN, *Virology*, 10 (1960) 384.
6 D. SHUGAR AND K. L. WIERZCHOWSKI, *J. Polymer Sci.*, 31 (1958) 269.
7 A. SIEGEL, S. G. WILDMAN AND W. GINOZA, *Nature*, 178 (1956) 1117.
8 A. D. MCLAREN AND W. N. TAKAHASHI, *Radiation Research*, 6 (1958) 725.
9 F. C. BAWDEN AND A. KLECZKOWSKI, *Nature*, 183 (1959) 503.
10 A. SIEGEL AND A. NORMAN, *Virology*, 6 (1958) 725.

Ultraviolet light sensitivity and other biological and physico-chemical properties of halogenated DNA

Since the methyl group of thymine apparently does not participate in any intra- or inter-strand bonding in the deoxyribonucleic acid (DNA) molecule, it can be replaced by halogens having similar van der Waals radii[1] (Cl 1.80, Br 1.95, CH_3 2.0, I 2.15 Å) or partial specific volumes* (CH_3 46.4, Cl 47.9, Br 69.3, I 83.4 cm^3). Thus bacteriophages[2], bacteria[3-5] and human cell lines[6-9] grown under conditions of thymine deficiency can incorporate 5-chloro-, 5-bromo-, or 5-iododeoxyuridine (CUDR,

* We are thankful to Prof. A. Wacker for these data calculated on the basis of the Leybold atomic models.

BUDR, or IUDR) into their DNA. CUDR and, to a slightly lesser extent, BUDR appear to be the best thymidine substitutes, as judged by their ability to promote the growth of 5-fluorodeoxyuridine (FUDR)-inhibited human D98/AG cells. Human cells in which BUDR[9] or CUDR has been substituted for 30 to 60% of the DNA thymidine are fully viable and can be cultivated indefinitely under these conditions. Bacteria (*Escherichia coli* and *Bacillus subtilis*) labeled in the same manner become very fragile, but the DNA of the latter appears to be fully functional, in terms of specific transforming activity for 3 auxotrophic markers, which was not altered when either one or both DNA strands were heavily BUDR-labeled (70% thymidine replacement)[10].

Despite its biological functionality, halogen-labeled DNA confers upon the organism a highly increased sensitivity to ultraviolet light (UV). In extreme cases, the survival rate for UV-irradiated BUDR-labeled human cells is the same as for unlabeled cells receiving a UV dose 20 times higher (20-fold dose reduction)[9]. With bacteria[11-12], phage ΦX-174[13], and intracellularly or extracellularly irradiated transforming DNA[14], radiosensitization corresponding to a 4- to 7-fold UV dose reduction is readily achieved with heavy BUDR labeling, while less than 2-fold sensitization is observed with extracellularly irradiated phage T_2[2,15]. The latter also becomes markedly more sensitive to visible light[15] as a result of BUDR incorporation.

BUDR-labeled human or bacterial cells are sensitized not only to UV but also to X-rays[9,17] and to the decay of DNA-incorporated ^{32}P, which kills cells stored in 10% glycerol at −70°, as observed by Dr. G. Ragni in our laboratory.

To effect maximum radiosensitization, both DNA strands must be BUDR labeled[9], *i.e.* two or more DNA replications must take place in the presence of BUDR. The extrapolation number characteristic for the UV-survival curve of cells containing only unifilarly labeled DNA (produced by one round of DNA replication after the addition or withdrawal of BUDR) is lower than that of unlabeled cells, although the final slope of the curve is not markedly altered[9]. Thus this radiosensitization phenomenon seems to be directly related to chemical modification of the DNA. This is especially apparent since CUDR, BUDR and IUDR, as thymidine analogues, are incorporated only into DNA, although there is a possibility that converted to ribotides they may also replace thymine ribonucleotides in soluble RNA[16] or in some hypothetical coenzymes, thus contributing to the lethal and growth-inhibitory effects which are particularly apparent with thymine analogue-treated bacteria.

Two general mechanisms may be proposed to account for the higher radiosensitivity of cells containing halogen-labeled DNA: (*1*) an intrinsic increase in the radiation lability of the labeled DNA; and (*2*) partial or complete loss of the ability of the DNA to undergo the process of spontaneous or enzymatic repair following irradiation damage. The experimental data are compatible with the simultaneous operation of both mechanisms.

(*1*) If the primary site of UV action on DNA is the thymine moiety[18], a greater UV sensitivity of 5-bromouracil as compared with thymine could account in part for the radiosensitizing effect of BUDR. Another basis might be the presence of weakened or ruptured nucleotide-to-nucleotide bonds wherever two adjoining thymidylic acid molecules were replaced by two BUDR nucleotides, creating excessive strains in the polynucleotide chain. The adjacent bases must be very tightly packed together, as in a "stack of pennies", since there is only 3.4 Å space for each nucleotide, while the "thickness" of an aromatic ring amounts to 3.7 Å[1]. Thus, it seems likely that any

References p. 545

additional electrostatic repulsion or a change in the size of the grouping on the purine or pyrimidine ring would tend to produce a strain or otherwise distort the double-helical rod-like structure of the DNA molecule. The existence of single-strand weaknesses in BUDR-labeled, fully transforming *B. subtilis* DNA could be inferred from measurements of the sedimentation constant of normal and labeled DNA ($S'_{20,w}$ = 44; corrected for a DNA concentration equal to 0), which roughly corresponds to a molecular weight of $30 \cdot 10^6$, as determined by extrapolating the data of Eigner[19] for native DNA and applying measurements of intrinsic viscosity. The latter value, however, for BUDR-labeled DNA amounts to only 100 dl/g (determined in 0.195 N Na+ at shear rates of 90, 60, 30 sec^{-1} and extrapolated to 0 shear; 25°; 5 μg DNA per ml), which corresponds to about half the expected value[19]. The intrinsic viscosity of native, unlabeled DNA with similar sedimentation characteristics is in the neighbourhood of 200 dl/g. Since the "melting profile" of the labeled DNA[20] does not suggest the presence of any denatured molecules, the data might be interpreted as indicating an increased flexibility of the BUDR labeled double-stranded molecules (as a result of single-strand weaknesses). Another observation which is compatible with this interpretation is the extensive degradation of BUDR-labeled DNA strands upon heating, as indicated by the equilibrium sedimentation pattern of heat-denatured, labeled DNA centrifuged in a CsCl gradient, showing wide spreading of the band in addition to an 0.015 g/cm^3 increase in the density; the latter is characteristic for the denatured DNA. This observation might also account for the higher sensitivity of BUDR-labeled bacteria to heat[11]. The data as a whole support the concept that faults exist in the longitudinal structure of BUDR-containing polymers, which conceivably could contribute to the increased lability to radiation. On the other hand, the strength of the hydrogen bonding between the polynucleotide strands of DNA would not seem to be affected by halogen labeling, since the "melting" temperatures of the labeled and unlabeled DNA measured at high ionic strength are similar[12]. At low ionic strength (0.001 *M* NaCl in 50% methanol solution) the "melting" temperature of brominated or iodinated DNA was actually 2 to 3° higher than that of non-labeled DNA extracted from *B. subtilis*. That hydrogen bonding is not involved in this radiosensitization process is also indicated by the high increment in the UV sensitivity of BUDR-labeled ΦX-174 phage[13], which contains single-stranded DNA.

The strongest support for the role of direct UV sensitization of halogen-labeled DNA comes from the experiments on the inactivation of CsCl-gradient-purified normal, BUDR-, and IUDR-labeled transforming DNA (*B. subtilis*, indole marker). The 37% "survivals" for these 3 types of DNA correspond to a UV dose of 5960, 1180 and 1430 $ergs/mm^2$ respectively. Since identical increments in radiation lethality were observed for both BUDR-grown intact cells and for BUDR-labeled transforming DNA extracted from these cells, it can be concluded that in the intact cell, DNA is the principal target of lethal radiation effects and thus the most radiosensitive of the indispensable cell components[14].

(*2*) The radiosensitizing effect of halogenation might also reflect a lower rate of spontaneous recovery of the labeled DNA, as exemplified by the decrease in photoreactivation of organisms containing BUDR-substituted DNA[11,12,15]. The halogen atoms lining the large groove of the DNA double-helix might conceivably interfere with the fit of the protein (enzyme) molecule active in the "dark" or "light" reactivation process, or with the protein "backbone" of the chromosomal structure. Irreversi-

bility of the UV-induced changes in BUDR as compared with reversibility of thymidine-localized UV damage can also be invoked to account for differences in the extent of photoreactivation.

BUDR labeling of DNA offers several opportunities for experiments on the fate and the mode of replication of DNA, since labeled molecules can be separated by the CsCl centrifugation procedure, and the cells are differentially marked by increased UV sensitivity. *E. g.*, using the "bifilarly" BUDR-labeled transforming DNA as a primer in the DNA synthesizing system containing DNA polymerase and "light" deoxynucleotide triphosphates, it would be possible to separate in the CsCl gradient[10] any newly synthesized "light" DNA (after at least two rounds of replication) and determine its transforming capacity, even though the latter were extremely low or extensively reduced by DNases as compared with that of the original "heavy" primer. Various other experiments of this type are feasible or have already been completed.

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1 L. PAULING, *The Nature of the Chemical Bond*, Cornell University Press, Ithaca, 1945, p. 189.
2 R. M. LITMAN AND A. B. PARDEE, *Biochim. Biophys. Acta*, 41 (1960) 117.
3 F. WEYGAND AND A. WACKER, *Z. Naturforsch.*, B7 (1952) 26.
4 S. ZAMENHOF AND G. GRIBOFF, *Nature*, 174 (1954) 306.
5 D. B. DUNN AND J. D. SMITH, *Biochem. J.*, 67 (1957) 494.
6 M. T. HAKALA, *J. Biol. Chem.*, 234 (1959) 3072.
7 M. L. EIDINOFF, L. CHEONG AND M. A. RICH, *Science*, 129 (1959) 1550.
8 W. SZYBALSKI AND B. DJORDJEVIC, *Genetics*, 44 (1959) 540.
9 B. DJORDJEVIC AND W. SZYBALSKI, *J. Exptl. Med.*, 112 (1960) 509.
10 W. SZYBALSKI, Z. OPARA-KUBINSKA AND E. EPHRATI-ELIZUR, *Federation Proc.*, 19 (1960) 306.
11 S. GREER, *J. Gen. Microbiol.*, 22 (1960) 618.
12 Z. LORKIEWICZ AND W. SZYBALSKI, *Biochem. Biophys. Research Communs.*, 2 (1960) 413.
13 A. W. KOZINSKI AND W. SZYBALSKI, *Virology*, 9 (1959) 260.
14 Z. OPARA-KUBINSKA, Z. LORKIEWICZ AND W. SZYBALSKI, *Biochem. Biophys. Research Communs.*, 4 (1961) 288.
15 F. W. STAHL, J. M. CRASEMANN, L. OKUN, E. FOX AND C. LAIRD, *Virology*, 13 (1961) 98.
16 H. S. KAPLAN AND P. A. TOMLIN, *Radiation Research*, 12 (1960) 447.
17 D. B. DUNN, J. D. SMITH AND P. F. SPAHR, *J. Mol. Biol.*, 2 (1960) 113.
18 R. BEUKERS AND W. BERENDS, *Biochim. Biophys. Acta*, 41 (1960) 550.
19 J. EIGNER, *The Native, Denatured and Renatured States of Deoxyribonucleic Acid (Ph.D. Thesis)*, Harvard University, Cambridge, 1960, p. 128.
20 J. MARMUR AND P. DOTY, *Nature*, 183 (1959) 1427.

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Photosensitization and protection from radiation by hematoporphyrin*

Any biological event produced by radiation is initiated by energy absorption. The absorption of energy is dependent on the type of radiation and on the substrate structure. In the case of a photodynamic effect there is a relationship between the energy absorption and the molecular structure of the sensitizer. Only the energy absorbed by the sensitizer is utilized, and generally the action spectra and the absorption spectra correspond. In the case of the effects produced by highly energetic radiation such as X-rays, any substrate can absorb radiation, independent of its molecular structure.

Recently[1,2] several attempts have been made to find out whether photosensitizers play a role under X-irradiation. In this connection, no definite conclusions have been reached; Schwartz[3] obtained inconclusive results in experiments carried out with porphyrins under X-irradiation. These experimental results suggest that it may be profitable to study the role of hematoporphyrin in the inactivation of an enzyme by visible light and X-rays in relation to the radiation dose and the substrate and hematoporphyrin concentrations.

Lysozyme (Armour) was used as a substrate. Any change in this well known crystalline protein can easily be detected by means of evaluation of the enzyme activity. Moreover, lysozyme has been extensively studied under photodynamic conditions by Shugar[4] and Weil[5]. These authors investigated the action of light on the activity of lysozyme in the presence of riboflavin and methylene blue. The enzyme activity was studied according to Caselli[6] by measurements of the optical density of *Micrococcus lysodeicticus* suspensions in the presence of different samples of the enzyme. Hematoporphyrin hydrochloride (Nordmark) was used in phosphate buffer at pH 8. Radiation sources were: a) a low pressure mercury lamp at a distance of 20 cm from the samples ($768 \cdot 10^2$ ergs/cm²/sec). b) an X-ray generator, 220 kV and 12 mA, at a distance of 25 cm (67 r/min exposure dose); an Al filter (0.1 mm) and a Cu filter (0.5 mm) were used.

Preliminary experiments showed that lysozyme irradiated with visible light in the presence of hematoporphyrin undergoes a loss in enzymatic activity. Even in the absence of hematoporphyrin there was a certain degree of inhibition depending on the time of irradiation. The photodynamic activity of hematoporphyrin was calculated from the difference of the inhibition obtained in the presence and in the absence of the dye. The photodynamic effect on lysozyme was studied as a function of the time of irradiation. Lysozyme (0.4 μg/ml) was irradiated in the presence of hematoporphyrin (10^{-5} M) for up to 180 sec.

In Fig. 1 the log of the % enzyme activity is plotted against the exposure time. The resulting straight line function shows that the reaction is of the first order.

In other experiments the phenomenon was studied as a function of the lysozyme concentration. In Fig. 2 the variations in the % enzyme activity after 60 sec of irradiation are plotted against the lysozyme concentration, from 0.1 μg/ml to 0.8

* This work was carried out during the tenure by one of us (A.C.) of a grant from C.N.R.N.

μg/ml. Hematoporphyrin concentration was constant (10^{-6} M). Fig. 2 shows that the inhibition increases with increasing concentration of the enzyme up to a maximum corresponding to 0.4 μg/ml and then there is a diminution of the effect.

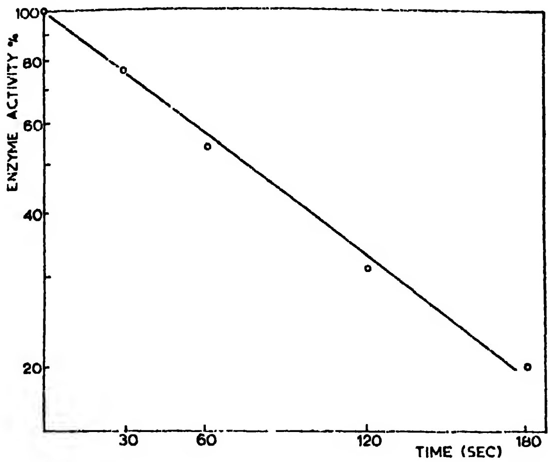

Fig. 1. Photodynamic inhibition of lysozyme as a function of time of irradiation.

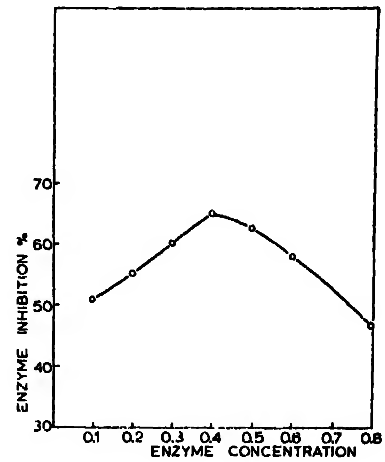

Fig. 2. Photodynamic inhibition of lysozyme as a function of enzyme concentration.

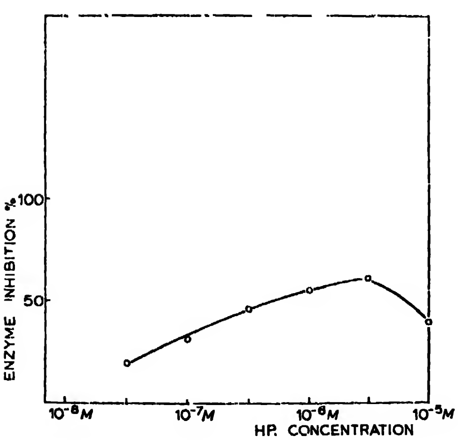

Fig. 3. Photodynamic inhibition of lysozyme as a function of hematoporphyrin concentration.

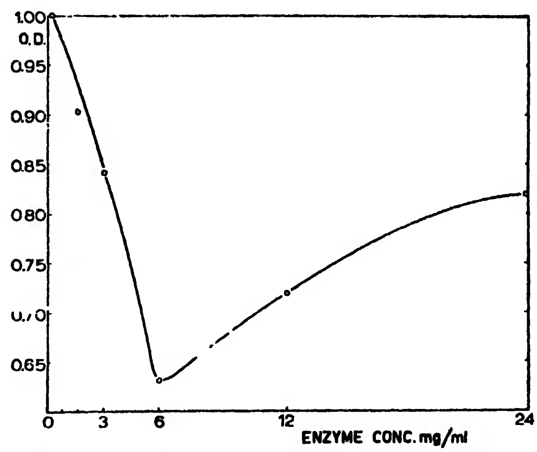

Fig. 4. Optical density variations at 395 mμ of the supernatant of hematoporphyrin solution ($10^{-6}$$M$) in the presence of increasing concentrations of lysozyme.

The photodynamic inhibition of lysozyme was also studied in relation to hematoporphyrin concentration. In Fig. 3 the % lysozyme inhibition (enzyme conc. 0.4 μg/ml) is plotted against the hematoporphyrin concentration from 10^{-8} M to 10^{-5} M.

Here also, as expected, the inhibition increases with increasing concentration of the hematoporphyrin up to a maximum and then decreases.

An objection could be advanced in this case, since the decrease of the effect after the maximum could be consistent with the filtering effect of the outer layer of the system at high concentration of the dye. However, the shape of the function is practically similar to that obtained in Fig. 2, where the concentration of hematoporphyrin was

constant. It seems that in our system there is a maximum of energy utilization when there is a certain hematoporphyrin/lysozyme concentration ratio.

Experiments carried out to verify this hypothesis showed the formation of a pink precipitate at pH 8.4 from a solution of hematoporphyrin (10^{-6} *M*) and lysozyme (6 μg/ml). With lysozyme at higher or lower concentrations, the precipitation took place to a lesser extent. This phenomenon was studied by measuring the optical density (395 mμ) of the hematoporphyrin solution of the supernatant of a series of testtubes containing increasing concentrations of lysozyme in the presence of hematoporphyrin at constant concentration (10^{-6} *M*). It is apparent from Fig. 4 that hematoporphyrin is bound to lysozyme in a definite ratio.

All the above mentioned experimental results are consistent with the fact that photodynamic inhibition of lysozyme is dependent on a combination of the dye with the substrate.

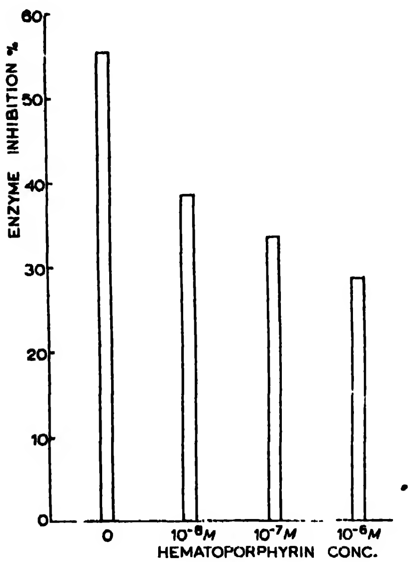

Fig. 5. Protective effect of hematoporphyrin on the inhibition of lysozyme by X-radiation. Enzyme conc. = 0.8 μg/ml. Irradiation = 2000 r.

The results of the attempts made in order to find out whether hematoporphyrin plays a sensitizing role when lysozyme is irradiated with X-rays, are shown in Fig. 5.

The presence of hematoporphyrin in the system under X-rays does not synergise the action of the high energy radiation; on the contrary, a definite protection was observed. The protective effect increased with the concentration of hematoporphyrin as shown in Fig. 5, where the % enzyme inactivation is plotted against the hematoporphyrin concentration.

To conclude: *(a)* lysozyme can be inactivated by photodynamic action of hematoporphyrin. *(b)* the photodynamic inhibition of lysozyme is dependent on a combination of the protein with the dye and shows a maximum at a definite concentration ratio of the two substances. *(c)* a protective action occurs when lysozyme is irradiated with X-rays in the presence of hematoporphyrin. This effect is dependent on the hematoporphyrin concentration.

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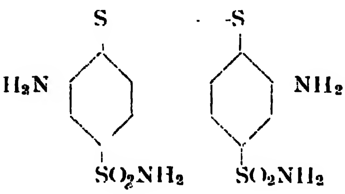
1 M. K. Loken, *Thesis*, University of Minnesota, 1956.
2 R. Koch, *Minerva med.*, 51 (1960) 34.
3 S. Schwartz, K. Absolon and H. Vermund, *Univ. Minn. Med. Bull.*, 27 (1955) 1.
4 D. Shugar, *Bull. soc. chim. biol.*, 33 (1951) 710.
5 L. Weil, A. R. Buchert and J. Maher, *Biochim. et Biophys. Acta*, 40 (1952) 245.
6 P. Caselli, *Boll. soc. ital. biol. sper.*, 22 (1946) 57.

Protective effects of some sulphanilamide disulphides on the inactivation by ultraviolet and Röntgen radiations of animal lactic and alcohol dehydrogenases*

The following substances and some other derived disulphides have been studied:



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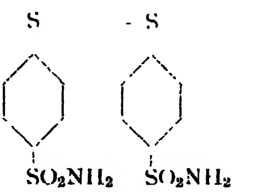

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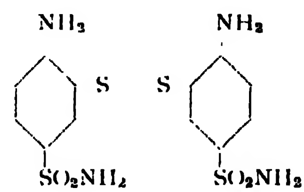

III

Most of the experiments deal with compound I, but all other disulphides give comparable results.

Previous experiments[1] have shown that these substances interact with yeast alcohol and lactic dehydrogenases. This interaction is shown by the appearance of two bands at 323 and 288 mμ, which are typical of the reduced compound. The interaction is of the type:

$$E\text{-}SH + R\text{-}SS\text{-}R \rightarrow E\text{-}SS\text{-}R + R\text{-}SH$$

with the subsequent possible reaction:

$$E\text{-}SH + E\text{-}SS\text{-}R \rightarrow E\text{-}SS\text{-}E + R\text{-}SH$$

Fig. 1 shows the band of the disulphide (line 1) and of its reduction product as obtained by addition of either cysteine (line 2) or yeast alcohol dehydrogenase (line 3). This interaction results in inactivation of the enzyme

However, these substances do not interact with all the -SH groups on a protein. Liver alcohol dehydrogenase (prepared according to Bonnichsen and Brink[2], and tested for activity according to Theorell and Bonnichsen[3]) is neither inactivated nor causes changes in the spectrum of the disulphide. Heart lactic dehydrogenase (prepared and tested according to Neilands[4]) shows this same behaviour. The substance must require special conditions on the protein surface in order that the interaction can take place.

I found that those enzymes which do not react with this substance are nevertheless protected from irradiation by it. This protection is evident both for irradiation with ultraviolet light and Röntgen rays. Dosimetry has been performed for ultraviolet

* This investigation was made possible by grants of the Comitato Nazionale per l'Energia Nucleare, Rome, and of the Rockefeller Foundation.

irradiation with a system containing ferric oxalate (according to Hatchard and Parker) and for Röntgen-rays with a system containing ferrous sulphate (according to Rösinger[6]). The extent of reduction and oxidation respectively were measured by means of o-phenanthroline and thiocyanate.

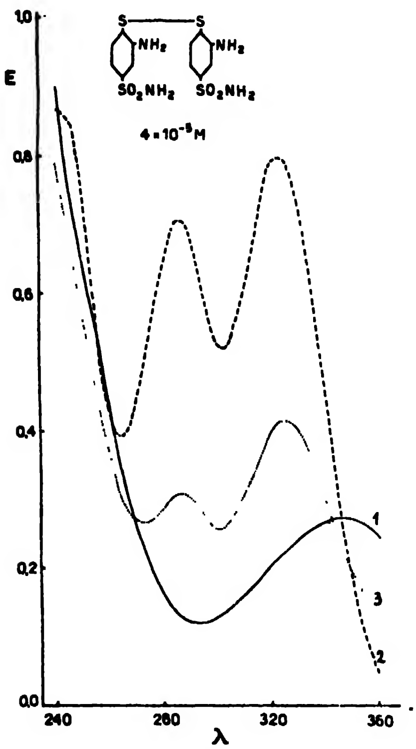

Fig. 1. Effects of cysteine and yeast alcohol dehydrogenase on the spectrum of the disulphide. 1, disulphide; 2, disulphide + cysteine; 3, disulphide + YADH.

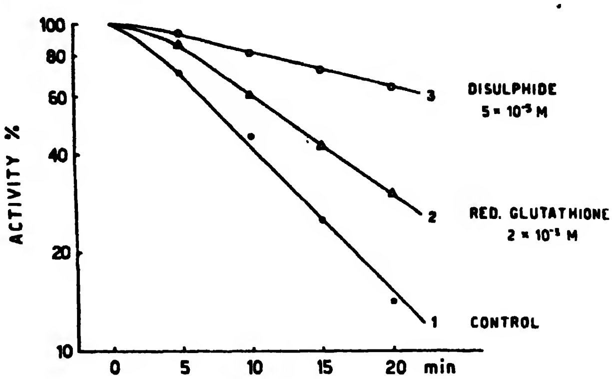

Fig. 2. Effect of reduced glutathione and disulphide on U.V.-irradiated heart lactic dehydrogenase.

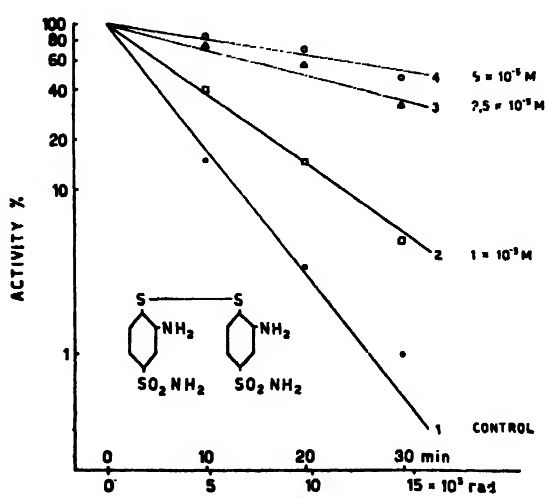

Fig. 3. Effect of disulphide on X-irradiated heart lactic dehydrogenase.

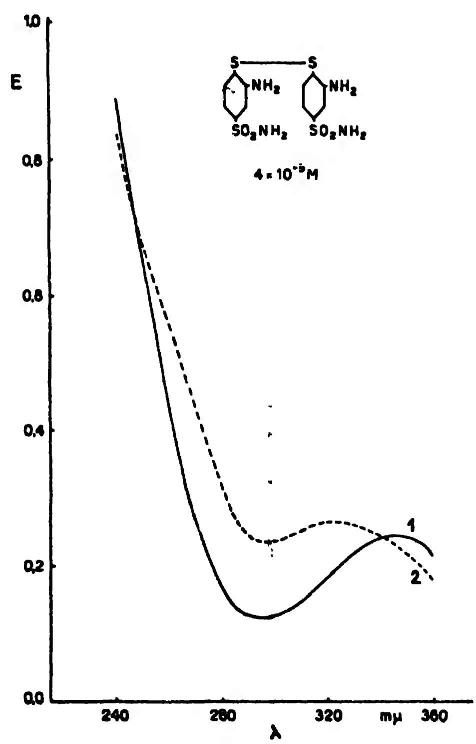

Fig. 4. Effect of ultraviolet light (2537 Å) on the spectrum of the disulphide. 1, not irradiated; 2, irradiated.

Fig. 2 shows the effect of U.V. irradiation (2537 Å; emitted by a generator Hanau NN 15/44) on heart lactic dehydrogenase in 0.01 *M* phosphate buffer pH 7.2. The ordinate is percent activity (logarithmic scale), the abscissa is time of irradiation. Line 1 shows the activity of enzyme alone; line 2 shows the effect of addition of reduced glutathione; line 3 shows the effect of addition of the disulphide 1. When liver alcohol dehydrogenase is irradiated with U.V. light in the presence of the disulphides, similar results are obtained.

Fig. 3 shows the protecting effect of the disulphide on the inactivation of heart lactic dehydrogenase in 0.1 *M* phosphate buffer pH 6, by X-rays produced by a Siemens apparatus at 120 kV, 20 mA, with aluminium filter of 2 mm (half-value layer of 0.18 mm Cu); irradiation distance, 9.5 cm. The energy absorbed by the solution under these conditions is 480 rad/min. Line 1 shows the inactivation of the enzyme alone, lines 2, 3 and 4 in the presence of various amounts of compound 1. It is evident that low concentrations of the disulphide are sufficient for a marked protection.

Similar protective effects of the disulphides are also observed with solutions of pancreas crystalline ribonuclease irradiated with ultraviolet light and X-rays[7].

Since the substances do not interact with these liver alcohol and heart lactic dehydrogenases and pancreas ribonuclease, an indirect effect was suggested. The substance possibly acts as a free radical trapping agent. In fact, the substance was also found to protect the enzyme against inactivation when incubated with a system which is known to produce free radicals, *e.g.* that formed by ferrous sulphate and hydrogen peroxide.

As I have observed it is remarkable that this substance has a much greater effect than cysteine or thiourea on the protection against ultraviolet irradiation. The quantum yield, calculated for the U.V.-irradiated heart lactic dehydrogenase and pancreas ribonuclease, showed that the protective effect does not depend on the U.V. light absorption on the part of the disulphides. On the other hand, for X-ray irradiation the effects of these substances are of comparable magnitude.

The protective effect is due either to the substance itself or to a derivative of it. Fig. 4 shows, in fact, that irradiation causes a change in the spectral properties of the substance.

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1 L. BRIGHENTI, *Boll. Soc. ital biol. sper.*, 35 (1959) 560, 1751.
2 R. K. BONNICHSEN AND N. G. BRINK, in S. P. COLOWICK AND N. O. KAPLAN (Editors), *Methods in Enzymology*, Vol. 1, Academic Press, 1955, p. 495.
3 H. THEORELL AND R. K. BONNICHSEN, *Acta Chem. Scand.*, 5 (1951) 1105.
4 J. B. NEILANDS, in S. P. COLOWICK AND N. O. KAPLAN (Editors), *Methods in Enzymology*, Vol. 1, Academic Press, 1955, p. 449.
5 G. C. HATCHARD AND C. A. PARKER, *Proc. Roy. Soc. (London)* A, 235 (1956) 518.
6 S. RÖSINGER, *Progress in Nuclear Energy, Series XII, Health Physics*, 1 (1959) 167.
7 L. BRIGHENTI AND A. FALASCHI, *Atti Simposium Radiobiologica Medica, Roma, 1960*, Comitato Nazionale per Energia Nucleare, Rome, (in the press).
8 G. PAPPALARDO, *Gazz. chim. ital.*, 87 (1957) 1484.

*Photosensitization by furocoumarins: psoralens**

This study concerns the biological photosensitization by a group of compounds called furocoumarins, more generally known as psoralens. Recently they have been widely used in augmenting the skin pigmentation of normal persons, for treatment of leukodermic skin and pigmentary disorders, in improving sun tolerance and prevention of skin cancers[1,2]. The biological photosensitization to long-wave ultraviolet light induced by psoralen (P) and 8-methoxypsoralen (8-MOP) and other related furocoumarins has revealed that (*a*) human skin as well as skin of several other mammalian species is photosensitized following topical or oral administration of the drug[3,4,5]; (*b*) bacteria and fungi are killed, seed germination is inhibited, and mutations in drosophilia are observed[6,7].

The purpose of this study was to explore the mechanism of psoralen photosensitization. The biological activity of these compounds was determined by: (*a*) an erythemal response of the skin observed visibly and photometrically by reflectance after 24 and 36 hours following topical application of these compounds on albino guinea pig skin[5]; (*b*) by photosensitized inhibition or inactivation of enzymes such as succinic dehydrogenase (SDH), lactic dehydrogenase (LDH) and cytochrome oxidase, both *in vivo* and *in vitro* systems. Mercury high pressure lamp emitting ultraviolet light of wavelengths >320 mμ was used. Absorption spectra of these compounds were obtained on Beckman recording spectrophotometer. The activating and the fluorescent wavelengths were determined by Aminco-Bowman spectrophotofluorimeter[8]. The action spectrum of psoralen was determined by studying the inhibition of LDH at different wavelengths from a monochromatic source in presence of psoralen.

The effect of structural alterations on the biological activity of furocoumarins with 36 furocoumarin and 42 coumarin derivatives was investigated. None of the compounds tested was more active than psoralen. Substitution with methyl groups at positions 4, 5′ and 8 did not reduce the activity but methyl substitution at 4′ or 3 positions significantly decreased the photosensitizing activity of psoralen. Simultaneous substitution at 3 and 4 positions also resulted in loss of activity. Substitution of an alkyl group larger than methyl decreased the activity. Substitution with methoxy, amino, nitro, acetyl, acetamino, bromo, ethylcarbamyl at 5 or 8 positions resulted in either partial or complete loss of activity. Oxazolocoumarin derivatives, isopsoralen derivatives were found to be inactive. Hydrogenation at 4′ and 5′ double bond practically eliminated the photosensitizing response. A benzo-difuran derivative was found to be active, but hydrogenation resulted in loss of activity. Most of the coumarin derivatives were inactive. In short, any other substitution or modification of psoralen structure was found to either lower the response or mitigate the activity.

The inhibition of SDH by psoralen or 8-MOP in presence of ultraviolet irradiation (>3200 Å) both *in vivo* (guinea pig skin) and *in vitro* system (rat liver mitochondria) and the inhibition of enzymes such as lactic dehydrogenase, cytochrome oxidase *in vitro* system was observed. The photosensitization by P or 8-MOP was independent of

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oxygen; in fact the presence of molecular O_2 decreased the degree of photosensitization. When O_2 was replaced by nitrogen or helium, inhibition of LDH or SDH could still be demonstrated. The temperature coefficient for 8-MOP photosensitization was less than 1.0. More enzyme activity was lost when illumination was carried at reduced temperature. The SDH and LDH enzymes were not protected against photosensitized inhibition by 8-MOP or P following the addition of SH-reagents such as cysteine, 2,3-dimercaptopropanol (BAL) and glutathione. Indeed, the addition of glutathione to rat liver mitochondria in progressively high concentrations (10^{-6} to 10^{-3} *M*) was found to increase the degree of photosensitized inhibition of SDH. Incorporation of ethylenediaminetetraacetate (EDTA $2 \cdot 10^{-3}$ *M*) into biological systems (SDH + psoralen, LDH + psoralen), prior to irradiation revealed a significant protection. No evidence was obtained which suggested that EDTA formed a complex with protein or its prosthetic group. No protective effect was observed when amino acids such as tyrosine, tryptophan, histidine, cysteine, methionine, and glutathione were incorporated in the biological system. Incorporation of quinones into LDH + psoralen system did not alter the photosensitization effect of P. However, proteins such as albumin and fibrinogen, vitamin B_{12} and DPNH prevented the photosensitization by P. The activating wavelengths for biologically active furocoumarins were found in two regions: (*1*) 265–280 mμ; (*2*) 340–380 mμ. The fluorescence peaks for these activating wavelengths were in the region of 420–460 mμ. The inactive furocoumarins did not reveal such relationship. It is more likely that furocoumarins which show

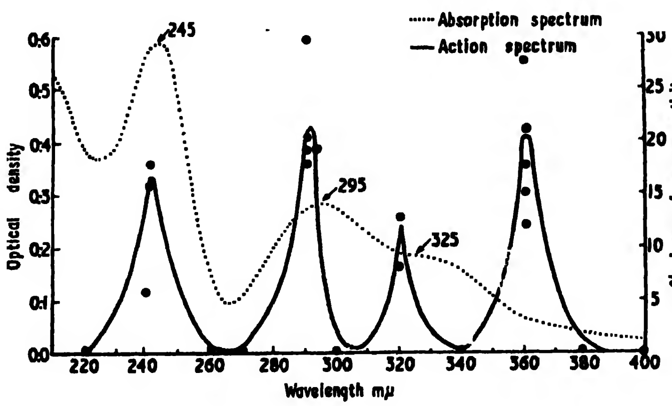

Fig. 1. Absorption spectrum and action spectrum of psoralen (photosensitized inhibition crystalline lactic dehydrogenase). ...... Absorption spectrum; —— Action spectrum.

activation peaks in the region of 340–380 mμ and concomitantly the fluorescent peaks in the region of 420–460 mμ can cause photosensitization of skin and inhibit enzyme activities in this region of long-wave ultraviolet light. The action spectra for these photosensitizing compounds lie in the region of 340–380 mμ. The action spectrum determined by the photosensitized destruction of LDH activity induced with psoralen was at 240, 290, 320 and 360 mμ. (See Fig. 1). This corresponded closely with the absorption spectrum of psoralen (245, 295 and 326 mμ). The 360-mμ wavelength which inhibited LDH in presence of P corresponded with the excitation peak (activating wavelength) of this molecule. It appears that the exciting wavelength of 360 mμ for maximum fluorescence of P represents a triplet state for this molecule,

References p. 554

which being highly reactive, induces biological changes. Two hypotheses are advanced: (1) It is postulated that the mechanism of P action involves a free radical formation in psoralen or psoralen plus protein mixture; (2) a metastable triplet state of psoralen has also been postulated which interacts and stabilizes free radical formed in proteins. It seems that the high energy and great reactivity associated with free radical or triplet state is responsible for photosensitized biological reactions induced by psoralens.

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[1] Proc. Symposium on Psoralens and Radiant Energy, *J. Invest. Dermatol.*, 32 (2) (1959) 132–301.
[2] M. A. PATHAK, F. DANIELS JR., C. E. HOPKINS AND T. B. FITZPATRICK, *Nature*, 183 (1959) 728.
[3] L. MUSAJO, G. RODIGHIERO AND G. CAPORALE, *Bull. soc. chim. biol.*, 36 (1954) 1213.
[4] T. B. FITZPATRICK, C. E. HOPKINS, D. D. BLICKENSTAFF AND S. SWIFT, *J. Invest. Dermatol.*, 25 (1955) 187.
[5] M. A. PATHAK AND T. B. FITZPATRICK, *J. Invest. Dermatol.*, 32 (1959) 509.
[6] W. L. FOWLKS, *J. Invest. Dermatol.*, 32 (2) (1959) 249.
[7] W. L. FOWLKS, D. G. GRIFFITH AND E. L. OGINSKY, *Nature*, 181 (1958) 57.
[8] M. A. PATHAK AND J. H. FELLMAN, *Nature*, 185 (1960) 382.

Zur Photoaktivierung des Provitamins D von Trockenhefe

Zur Bestrahlung von *Torula utilis* in Pulverform auf einem Transportband wurde das Gesamtspektrum des Hg-Hochdruckbrenners PRK2 verwendet[1]. Die oberste Hefeschicht, die der UV-Quelle am nächsten liegt, absorbiert weitgehend Strahlung und wirkt als Filter für die tiefer liegende Hefe. Deshalb musste die auf dem Transportband in einer Schichtdicke von 0.5 mm aufgetragene Hefe nach Passage des Strahlenkegels jedes Brenners durch Leitrollenpaare gewendet werden. Auf diese Weise wurde in *Torula utilis* — abhängig vom Steringehalt — maximal ein Vitamin D-Gehalt von

Hefe	*Steringehalt*	*I.E. Vitamin D maximal pro Gramm Hefe*
Torula utilis	0.2 %	12 000
Torula utilis	0.6 %	40 000

erzeugt.

Diese Ergebnisse veranlassten zur Bestrahlung von Backhefe mit hohem Steringehalt. Da mit Anstieg des Steringehaltes zumeist auch ein erhöhter Fettgehalt verbunden ist, musste ein anderes Bestrahlungsverfahren herangezogen werden[1].

Innerhalb einer Trommel befindet sich ein Reflektor, unter dem die entsprechenden Hg-Dampfdrucklampen angebracht sind. Die Trockenhefe wird in die Trommel eingeschüttet und breitet sich bei Drehung der Trommel auf einem Teil der Innenwandung aus. Durch andauernde Drehung der Trommel wird die Hefe umgewendet. Die an der Trommelinnenwand haftende Hefe wird durch eine Bürste abgestrichen.

Der Grad der Fotoaktivierung des Provitamins D wurde durch Fällung des nichtaktivierten Sterins mit Digitonin in dem zu verschiedenen Zeitabschnitten entnommenen Hefeproben bestimmt. Die Feststellung der antirachitischen Wirksamkeit erfolgte im prophylaktischen Röntgentest, da die meisten chemischen Vitamin D-

Bestimmungsmethoden unzuverlässige Werte ergaben[1,2]. Die Hefeteilchen wiesen eine Grösse von 0.15–0.3 mm auf. Bei Bestrahlung grösserer Hefeteilchen wurde nur ein geringerer Aktivierungsgrad erreicht, bei Verwendung kleinerer Teilchen verhinderte Staubentwicklung ein ordnungsgemässes Bestrahlen.

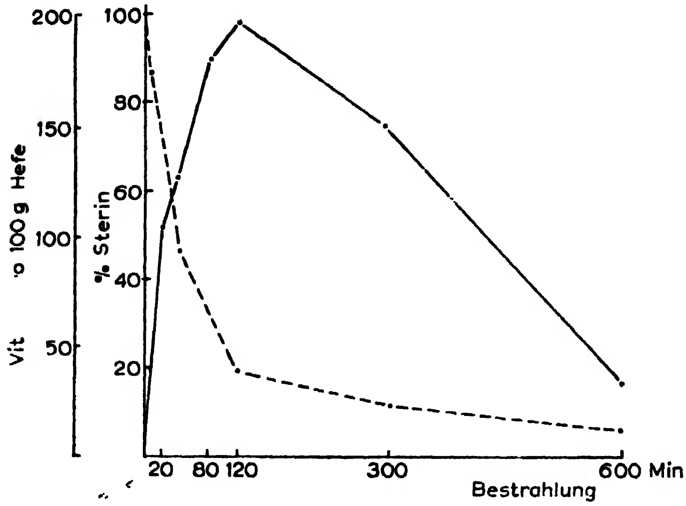

Abb. 1. Ergosterin-Isomerisierung und Vitamin-D Bildung in hellgelber Trockenbackhefe (Teilchengrösse der Hefe 0.15–0.3 mm). - - - - Sterin; —— Vitamin D.

Das Diagramm zeigt ein asymptotisches Verschwinden des mit Digitonin fällbaren Sterins innerhalb 10 Stunden. Die antirachitische Wirksamkeit steigt bis zu einem Maximum und fallt bei weiterer Bestrahlung stark ab -- verhält sich also ähnlich, wie die Vitamin D Aktivität bei Bestrahlung reinen Ergosterins[3].

Bei äquivalenten Strahlungsfluss der Resonanzlinie 254 mμ (Niederdruckbrenner NK 25/85) wird ein gleichartiges Verhalten sowohl in bezug auf Isomerisierung des Digitonin fällbaren Sterins als auch auf Vitamin D-Bildung beobachtet.

Ähnlich starke Vitamin D-bildende Effekte der Wellenlänge 254 mμ konnte ich gemeinsam mit Pfennigsdorf[1,4,5] bei Bestrahlung von Ratten und von Milch feststellen; Pfordte[3] beobachtete diesen Effekt bei Ergosterinaktivierung.

Interessant ist in diesem Zusammenhang, dass die antirachitische Wirksamkeit bestrahlter Hefe relativ lange konstant bleibt. So zeigten Hefeproben mit 48000 I.E. Vit. D/g nach sechsmonatiger Aufbewahrung bei 40° im Brutschrank keine Abnahme der Vitamin D-Wirksamkeit, während kristallisiertes Vitamin D unter diesen Bedingungen zerstört wird. Auf Grund entsprechender Modellversuche kann angenommen werden, dass ein mechanischer O_2-Schutz der Hefezellwand grossen Einfluss auf die Stabilität des aktivierten Ergosterins besitzt. Vielleicht liegt auch ein Teil des Vitamin D im Hefezellkomplex in Bindungen vor, die eine rasche Zerstörung unmöglich machen.

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1 W. PONSOLD, *Habilitationsschrift*, Halle, 1957.
2 H. BEKEMEIER UND W. PONSOLD, *Pharmazie*, 15 (1960) 543.
3 K. PFORDTE, *Z. physiol. Chem.*, 312 (1958) 193.
4 W. PONSOLD UND G. PFENNIGSDORF, *Arbeitstagung Biophysik*, Greifswald, Berlin, 1954, S. 70.
5 G. PFENNIGSDORF, W. PONSOLD UND E. O. SEITZ, *Strahlentherapie*, 94 (1954) 137.

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Session 6

PHOTOBIOCHEMISTRY (ESPECIALLY PIGMENTS) AND MICROBIOLOGY

Chairman: AMLETO CASTELLANI, Milan (Italy)

Secretary: FREDERIK ENGEL, Copenhagen (Denmark)

The mechanism of the reversible photoreduction of porphyrins

Work carried out in the Laboratory of Photobiochemistry, Institute of Biochemistry, U.S.S.R. Academy of Sciences, showed that compounds of the porphyrin or chlorin type, as well as their complexes with some metals (Mg, Zn) possess the ability to undergo reversible photochemical reduction[1-3]. Upon illumination of the solutions of these compounds in the presence of various reducing agents under anaerobic conditions, reduced products are formed that have a higher reducing potential than the original ones. This shows that it is possible to store up the energy of the absorbed light in the compounds formed.

The reaction mechanism of photoreduction of the compounds of porphyrin or chlorin structure is of considerable interest since several biologically important substances, the group of chlorophylls in particular, have a closed tetrapyrrol ring, and the photosensitizing action of these substances may be related to their capacity to rapidly accept and give up an electron.

Photoreduction of the compounds mentioned under usual conditions leads, as a rule, to the formation of relatively stable reduced products which undergo the reverse reaction relatively slowly in the dark, even when oxygen is admitted; these products can hardly be regarded as appropriate ones for the role of intermediates, *i.e.* electron carriers in the sensitization of redox reactions. We found that the reduced products formed under usual conditions are not the primary products of photochemical reduction of pigments and that their appearance in solution is preceded by the formation of much more labile reduced products[3]. We succeeded in this by lowering the temperature of the experiment to —45°, —65°. As a result, the secondary reaction of photoreduction which is usually observed at room temperature was strongly decelerated. The primary photoreduced product of metal-containing porphyrins and chlorins, in particular chlorophylls, turned out to be very unstable, so that its presence could only be detected by means of electrometrical measurements[4]. By contrast, a similar product formed by metal-free pigments (*e.g.*, pheophytins, protopheophytin, haematoporphyrin) is rather stable at low temperature, and we succeeded in recording spectral changes corresponding to the appearance of this product and, in some cases, even in measuring its complete absorption spectrum[5,6].

The relative stability of the primary products of metal-free pigments at low temperature enabled us to follow the kinetics of the formation and back reactions of these compounds under various conditions and to study some of their properties. It turned out that the velocity of their formation, unlike that of the formation of the secondary compounds, is temperature independent within the range —20° to —100°[7]. This does not change when usual reducing agents are replaced by deuterated ones[8].

Electrometrical measurements of changes of the redox potential and the electroconductivity of photochemically reacting solutions have shown that the observed changes of the potential and the conductivity are determined by the appearance in

References p. 561

the solution of the primary reduced product and that only this product is active[4] on the electrode, and, apparently, ionized.

The primary reduced products of all the compounds studied are very reactive and react rapidly with the electron acceptors possessing a normal redox potential more positive than −0.32 to −0.34 V (*e.g.*, safranine T, neutral red, diphosphopyridine-nucleotide) even at a very low temperature (in our experiments, down to −120°).

The properties of the primary reduced product indicate that it appears as a result of electron transfer from the reducing agent to the pigment and that it is a compound of the type known as an ionized free radical, *i.e.* a semiquinone. The secondary reduced form produced by photochemical reduction of pigments at room temperature seems to be a result of the dark addition of a proton to the primary semiquinone. The probability that the primary photoreduced form is a free radical found corroboration in further work of our laboratory on the measurement of electron paramagnetic resonance spectra and in experiments on the initiation of methylcrylate polymerization upon the photoreduction of pigments[9].

The properties of the primary reduced product, as a free radical, allow us to regard it as appropriate for the role of an intermediate electron carrier in photosensitization by pigments of oxydoreductive reactions. The mechanism of such sensitization seems to be the following: a pigment molecule in the long-lived excited state accepts an electron (or some electrons?) from the electron donor, as a result of which the primary reduced form of the pigment is formed. This reacts with the acceptor and transfers an electron to it. The original pigment is thus regenerated, and the semiquinone of the acceptor is formed which can either dismutate or subsequently add a proton. The whole process can take place very quickly, particularly when it proceeds within the complex formed between the pigment, the donor, the acceptor and, presumably, the solvent.

The possibility of this kind of sensitization mechanism for metal-free compounds of porphyrin or chlorin structure was confirmed by us by means of the separation of the processes of the formation of the primary reduced form and those of its reaction with the electron acceptor. For the compounds containing a metal atom in the centre of the molecule this could be done by means of electroconductivity measurements. Rapid changes of the conductivity under the alternation of light and darkness at low temperature which are characteristic of pyridine pigment solutions (*e.g.*, those of chlorophyll) and are due to a rapid appearance and disappearance of the labile ionized reduced form, cease in the presence of the non-reduced acceptor and are resumed again on its complete reduction[10].

The above described mechanism of photoreduction and photosensitization is characteristic of tetrapyrrol compounds with a closed ring of conjugated double bonds.

Special experiments[11] have shown that tetrapyrrol compounds with an open ring, bilirubin in particular, lack the ability to undergo rapid photochemically reversible reduction and that the efficiency of their sensitizing action is much less than that of porphyrins and chlorins.

It is quite possible that the mechanism of photosensitization by porphyrins and chlorins which we have put forward for solutions and which includes the formation of the primary reduced form also takes place under heterogeneous conditions[12] and in living organisms. In particular, such a mechanism is very likely to hold for the chlorophyll on the sensitization of photosynthesis. The rather high reactivity and

lability of the primary reduced form of this pigment seems to provide the high efficiency of the sensitizing process, and, therefore, the efficient utilization of the light energy absorbed.

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1 A. A. Krasnovsky, *Doklady Akad. Nauk S.S.S.R.*, 60 (1948) 421.
2 A. A. Krasnovsky, *Zhur. Fiz. Khim.*, 30 (1956) 968.
3 V. B. Evstigneev, *Zhur. Fiz. Khim*, 32 (1958) 969.
4 V. B. Evstigneev and V. A. Gavrilova, *Doklady Akad. Nauk S.S.S.R.*, 103 (1955) 97.
5 V. B Evstigneev and V. A. Gavrilova, *Doklady Akad. Nauk S.S.S R.*, 96 (1954) 1201.
6 V B. Evstigneev and V. A. Gavrilova, *Doklady Akad. Nauk S S.S.R.*, 118 (1958) 1146.
7 V. B. Evstigneev and V A. Gavrilova, *Doklady Akad Nauk S S S.R.*, 114 (1957) 1066.
8 V. B. Evstigneev and V. A Gavrilova, *Doklady Akad. Nauk S.S.S R.*, 115 (1957) 530.
9 A. A. Krasnivsky and A. V Umrikhina, *Biofizika*, 3 (1958) 547.
10 V B. Evistigneev and V. A. Gavrilova, *Doklady Akad. Nauk S.S.S.R.*, 127 (1959) 198.
11 V B. Evstigneev and V. A. Gavrilova, *Biofizika*, 4 (1959) 641; *Doklady Akad. Nauk S.S.S.R.*, 126 (1959) 410

Reversible photoreduction of porphyrins and the mechanism of photosensitization

The ability of chlorophyll and its analogues to undergo reversible photochemical reduction underlies the photosensitizing action of these pigments in the processes of photochemical hydrogen (electron) transfer[1]. The present communication briefly summarizes investigations carried out in our laboratory on the photochemical reduction of porphyrins, which we discovered several years ago. The reaction is closely related to the photosensitizing (photodynamic) properties of these pigments.

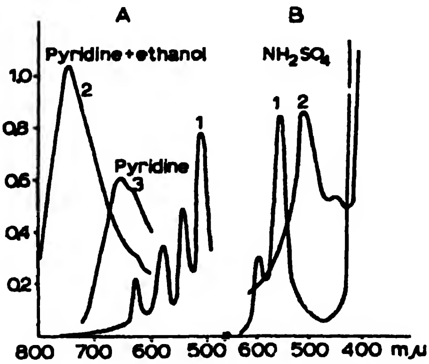

Fig. 1. Reversible photoreduction of hematoporphyrin by ascorbic acid in different media. 1 — before illumination and after reverse reaction; 2, 3 — after illumination.

Upon illumination of the porphyrin solution in the presence of electron donor molecules in vacuo, the absorption spectrum of the solution changes as a result of the formation of photoreduced pigment with specific spectral properties (Fig. 1). When the light is switched off, the reverse reaction is accelerated in the dark by admission

References p. 564

of air, oxygen or some electron-accepting molecule (oxidizing agent). By this means, the photoreduced pigment is reversibly oxidized and the initial porphyrin spectrum is restored. Hematoporphyrin, photoporphyrin and porphyrins of photosynthesizing bacteria were studied in our laboratory[3]. The photochemical properties and absorption spectra of these pigments are similar but differences in the velocity of photoreactions and back processes were observed in different pigment species. In most experiments we studied the porphyrin–ascorbic acid system. We observed porphyrin photoreduction in all media where pigment and electron donor were soluble, but the properties and reactivity of photoproducts greatly depend on the acid–base properties of the medium[4].

In strongly basic media *e.g.* piperidine and pyridine, photoreduced pigments having a main absorption maximum at 640–660 mμ are formed. Dr. Evstigneev from our laboratory observed that if reaction takes place in pyridine at —40°, the formation of active reduced product with absorption maxima at 460 mμ predominates; this intermediate reacts rapidly with atmospheric oxygen[5]. In basic media the protons of porphyrin carboxylic groups and electron-donating molecules are bound to molecules of medium. The source and the initial result of the photochemical process is the electron transfer from molecule (or ion) electron donor to the excited porphyrin molecule. In alcohol or water containing 10% of pyridine, photoproducts with an absorption maximum at 740 mμ are formed; in this case the carboxylic group dissociation is probably not complete. Finally in acidic aqueous solutions (2 *N* sulphuric acid) we demonstrated the formation of photoreduced porphyrin with the main absorption maximum at 520 mμ[6]. In this case positive porphyrin ions accepting one electron are probably formed, the carboxylic group dissociation is completely inhibited and the central nitrogen atoms of the porphyrin ring are bound to protons. We also observed the fluorescence of photoreduced porphyrin with a maximum at 760 mμ, probably corresponding with the form with absorption maximum at 740 mμ.

It is important to state that the shift of acid–base properties of the medium leads to a reversible shift of the absorption spectra of the photoreduced porphyrins[4] but that in all cases the pigment regenerates after air admission. In all cases we observed a negative shift of the potential of an inert platinum electrode immersed in the photo-

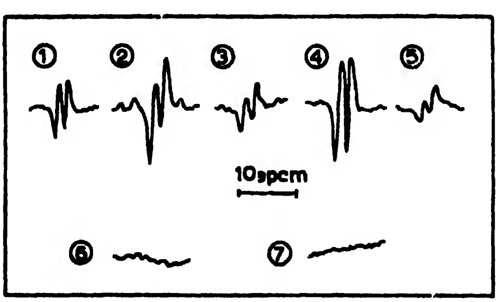

Fig. 2. ESR spectrum during illumination of the pigment–ascorbic acid system *in vacuo*. 1. chlorophyll *a* 2. chlorophyll *b* 3. pheophytin 4. hematoporphyrin 5. magnesium phthalocyanine 6. without pigment 7. without electron donor.

reacting system of pigment and electron donor. This indicates the formation of negatively charged electrode-active photoproducts.

In agreement with the general photoreduction scheme shown below, the primary

act of the reaction is the electron acceptance by the excited porphyrin molecule (P) from the electron-donating molecule (AH). The excited pigment probably reacts while in a long-lived metastable triplet state. The primary photoreaction results in ion-radical pair formation:

$$P + AH \rightleftharpoons P\,AH \rightleftharpoons \cdot P^- + \cdot AH^+$$

Our experiments revealed that the porphyrin–electron donor system is capable of initiating methyl-methacrylate polymerisation under visible light illumination; this indicates free radical formation during the reaction[7]. The measurement of electron spin resonance absorption spectra of the hematoporphyrin–ascorbic acid system in pyridine by its direct illumination in the resonator of the spectrometer revealed the appearance of an ESR spectrum which disappeared after the light was switched off[8]. The fine structure of the ESR spectrum corresponds to that observed during illumination of ascorbic acid in piperidine and it is probably that of the monodehydroascorbic acid radical. The ESR spectrum of the reduced pigment radical which is formed during the reaction has not yet been observed because of the intense background signal of the oxidized ascorbic acid radical (Fig. 2).

To sum up, all the experimental data available lead to the conclusion that the mechanism of porphyrin photoreduction consists of the oxido-reduction stage of electron transfer accompanied by a new acid-base equilibrium which depends upon the properties of the medium and the electron-accepting pigment. The photosensitizing ability of porphyrins for oxidation of some hydrogen donors was known long ago, but the mechanism of the reaction was not definitely elucidated (Fig. 3). On the basis of our experiments with porphyrin photoreduction, we can conclude that this reaction gives rise to the porphyrin photosensitizing ability. The following observations support this conclusion:

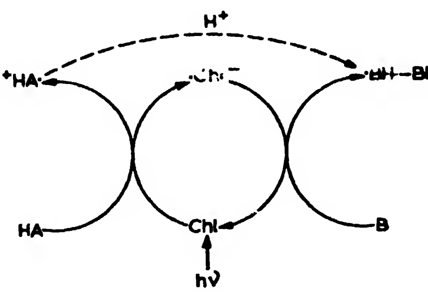

Fig. 3. Mechanism of photosensitizing action of porphyrins and analogues. Path of electrons: — -- Path of protons: - - -

(*1*) Photosensitized oxido-reduction occurs only with those hydrogen (electron) donors which are capable of photoreducing the excited pigments.

(*2*) In the triple system, pigment–electron donor–electron acceptor, the formation of photoreduced pigment cannot be observed until all the electron acceptor present has been reduced as a result of sensitized reaction.

(*3*) Finally, one can separately observe the stages of photoreduced pigment formation and the "dark" reaction of these with oxygen (or other electron acceptor) proceeding with regeneration of original pigment.

References p. 564

The photosensitized reaction consists, therefore, of a stage of pigment photoreduction followed by "dark" stages of its interaction with an electron acceptor — there is a possibility that in this case a semioxidized electron donor molecule readily reacts with oxygen. This results in pigment regeneration and sensitized oxidation of the electron-donating molecule.

Blum[9] showed that the photodynamic action of porphyrins and various dyes is revealed in the presence of oxygen, and is probably due to random destructive oxidation of vital metabolites and proteins. By this means, oxidation of side chain amino acids, such as cysteine, hystidine etc. may occur, thus inactivating enzymes and proteins. In addition oxidation of the components of biochemical oxido-reductive systems (dienols, quinones and similar compounds) may take place.

On the basis of the experiments described we can put forward the hypothesis that the photodynamic action of porphyrins and other dyes is due to their ability to undergo reversible photochemical reduction.

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1 A. A. KRASNOVSKY, *J. Chim. Phys.*, 55 (1958) 968, *Uspekhi Khim.*, 29 (1960) 736.
2 A. A. KRASNOVSKY AND K. K. VOINOVSKAYA, *Doklady Akad. Nauk S.S.S R.*, 96 (1954) 1209.
3 K. K. VOINOVSKAYA AND A. A. KRASNOVSKY, *Biokhimiya*, 20 (1955) 123.
4 A. A. KRASNOVSKY AND E. V. PAKSHINA, *Doklady Akad. Nauk S S.S.R.*, 120 (1958) 58.
5 V. B. EVSTIGNEEV AND V. A. GAVRILOVA, *Doklady Akad. Nauk S.S.S.R.*, 118 (1958) 1146.
6 A. A. KRASNOVSKY AND A. V. UMRIKHINA, *Doklady Akad. Nauk S S.S.R.*, 122 (1958) 1061.
7 A. A. KRASNOVSKY AND A. V. UMRIKHINA, *Biofizika*, 3 (1958) 2.
8 N. N. BUBNOV, A. A. KRASNOVSKY, A. V. UMRIKHINA, V. TSEPALOV AND V. JA. SHLAPENTOKH, *Biofizika*, 5 (1960) 121.
9 H. BLUM, *Photodynamic Action and Diseases Caused by Light*, Interscience, New York, 1941.

Étude par fluorescence des porphyrines concentrées dans les éléments minéraux biologiques

Un minéral particulier, la quincyte, est un silicate de chaux et de magnésie coloré en rose et doté d'une nette fluorescence rouge sous les rayons ultraviolets filtrés de la région 3.600 Å. L'étude de ce minéral nous a montré que cette fluorescence était due, de même que celle de certaines silices hydratées roses de la même région, à des concentrations de porphyrines[1].

D'où viennent ces éléments biologiques dans ces roches sédimentaires de la localité de Quincy? Peuvent-elles être le fait d'animaux vivants aux époques géologiques? A vrai dire cette question pouvait de prime abord sembler saugrenue car il est connu que si les porphyrines se manifestent *in vivo* par des concentrations dans certaines parties de l'organisme, et, en particulier, lors de certaines calcifications, elles sont en ces conditions fort peu stables.

Un exemple bien connu est celui qui se manifeste avec la fluorescence des oeufs de poules, canards . . . Frais pondus, ceux-ci montrent en lumière de Wood une très vive fluorescence rouge pourpre due à une localisation superficielle de porphyrines mais cette propriété optique disparaît très vite à la lumière ou à l'ultraviolet[2].

Par contre des observations ont aussi été faites relativement à des concentrations *in vivo* de porphyrines dans des organismes vivants, lors de certaines calcifications en particulier. C'est ainsi que des taches fluorescentes rouges sur des ossements apparaissent en liaison avec une lente agonie de l'individu dans un lieu obscur. L'identification de la porphyrine dans de telles conditions peut se faire avec une précision extrême puisque la fluorescence se manifeste vivement pour des concentrations de l'ordre de 1 partie par milliard en solution. Ici la solution se trouve faite en solution solide dans les parties calcifiées.

Ors un être humain, par exemple, fixe puis élimine constamment des porphyrines. L'urine normale en contient de 10 à 200 $\mu g/l$ et nous éliminons environ un quart de milligrammes de coproporphyrine outre un peu de protoporphyrine dans les fèces. Mais cette présence est constante dans tous les êtres vivants puisque ce sont les complexes de la protoporphyrine avec le fer, combinés à des protéines spécialisées, qui fournissent les cytochromes respiratoires présents dans toutes cellules vivantes.

L'utilisation des isotopes indicateurs ont permis de nouvelles investigations sur la synthèse des porphyrines chez les animaux et sur la nature des aberrations qui sont à l'origine des redoutables porphyries[3]. On sait que du fait de la forte photoactivité des porphyrines cette maladie provoque une intense sensibilisation à la lumière. Des ossements, des dents d'animaux atteints de porphyrie montrent des taches fluorescentes très caractéristiques. Ors la fluorescence des porphyrines est elle-même facile à définir par les bandes types[4].

Reprenant des observations parallèles nous avons pu retrouver des fluorescences de ce type d'abord sur des ossements datant d'il y a plusieurs années. Un cas assez typique est celui des ossements du chien de l'Aven Marzal. Puis sur des ossements d'époques préhistoriques (dolmens Poitevins).

Dès lors la présence de porphyrine concentrée *in vivo* en des dépôts sédimentaires est fixée en forme de solution solide dans un calcaire ou un silicate et stabilisée ainsi n'apparait plus aussi mystérieuse et paradoxale, et le fait de la quincyte, s'il demeure exceptionnel, mérite d'être retenu comme un caractère naturel. On la retrouvera peut-être chez certains animaux marins ou colonies à calcifications car déjà ont été cités des cas de dépôts d'uroporphyrines dans des coquilles marines.

Nous ne sommes pas loin, ici, de fermer un cycle naturel avec des dépôts sédimentaires cependant que les troublantes questions posées par les fluorescences de certaines colonies* peuvent trouver là une base d'étude rationnelle.

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[1] M. DÉRIBÉRÉ, *Comm. Sté Photobiologie*, vendredi 14 novembre 1958.
[2] M. DÉRIBÉRÉ, *Les applications pratiques de la luminescence*, 3ème éd., Dunod, Paris, 1955.
[3] C. REMINGTON, *Endeavour*, 55 (1955); D. SHEMIN ET D. RITTENBERG, *J. Biol. Chem.*, (1946) 621; D. SHEMIN ET J. WITTENBERG, *J. Biol. Chem.*, (1951) 315.
[4] CH. DHÉRÉ, *La fluorescence en biochimie*, Presses Univ. France, 1937.

* Coraux fluorescents du DR. CATALA par exemple.

Pigments and photoresponses in fungi

Phototropic and photomorphogenetic responses are widespread in fungi, and the photosensitive organs often contain carotenoids in amounts easily observable by the eye. This has led to a widespread view that carotenoids are the photoreceptor pigments of fungi. The validity of this concept has been examined by means of experiments on two species in which the relationship between carotenoid production and photomorphogenetic effects seemed particularly striking.

In *Fusarium oxysporum*[1], daylight brings about both carotenoid formation and greatly enhanced asexual reproduction, numerous macrospores being produced instead of the small number of microspores characteristic of cultures grown in darkness. It was found, however, that although visible light is effective in promoting carotenoid formation, wavelengths greater than 3600 Å had only feeble effects on reproduction. Shorter wavelengths were highly effective in promoting macrospore formation and by adjustment of wavelength and dosage it was possible to obtain carotenoids in the absence of macrospores, or macrospores in the absence of carotenoids. It is therefore clear that in this organism the photomorphogenic response can occur in the absence of carotenoids, and that some other photoreceptor substance must be sought.

In *Pyronema confluens*[2] daylight brought about carotenoid formation and sexual reproduction. It was found that visible light was effective in promoting both these responses. An albino mutant was obtained, however, which was unable to respond to illumination by forming carotenoids but which nevertheless needed light for reproduction. In this species also, therefore, carotenoids cannot be the photoreceptor substances in the photomorphogenetic response.

Various workers are now studying photoresponses in many species of fungi, a particularly active field being the study of phototropism in *Phycomyces* and *Pilobolus*. Results obtained by a variety of methods seem to exclude the possibility of a carotenoid photoreceptor and to indicate flavins as a possible, and perhaps the only, photoreceptor for phototropic and photomorphogenetic responses to visible light in fungi. Photomorphogenetic effects confined to U.V. wavelengths are limited to a few species such as *Fusarium oxysporum*; here the possibility of a pteridine photoreceptor requires investigation.

The rôle of carotenoid pigments in fungi remains unsolved. Carotenoids, as indicated above, are commonly abundant in photosensitive organs and are often light-induced. It has been shown in several species of bacteria that carotenoids protect the organism from damage by intense light, although the mechanism of the effect is imperfectly understood. It seems possible that the fungal carotenoids have a similar function.

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[1] M. J. CARLILE, *J. Gen. Microbiol.*, 14 (1956) 643.
[2] M. J. CARLILE AND J. FRIEND, *Nature*, 178 (1956) 369.

Photochemical conversion of protochlorophyll to chlorophyll-*a*

In dark-grown seedlings of wheat the synthesis of chlorophyll-*a* cannot be completed without a supply of light-energy. One of the last steps in the biosynthesis of chlorophyll-*a* is the reduction of protochlorophyll to chlorophyll-*a*. In wheat and most other higher plants this process requires light.

Previous studies of this photochemical process have shown that the rate of this reaction follows the course of a second-order reaction with respect to concentration of protochlorophyll. This fact has led to the assumption that the conversion of protochlorophyll is not strictly a photochemical process. It has been proposed that the reaction might be bimolecular and that some enzymatic reactions could be involved. In most of the experiments on this process, long periods of illumination are used, often up to several minutes. Now, if considerable time elapses between the beginning of illumination and the extraction, some enzymatic reactions might take place unnoticed.

In my experiments I have tried to shorten the illumination time. I found that when the light intensity is sufficiently high it is possible to transform 40–50% of the initial amount of protochlorophyll in wheat leaves to chlorophyll-*a* using an illumination time of 1/2,000 sec. Fig. 1 shows some results with etiolated wheat leaves illuminated

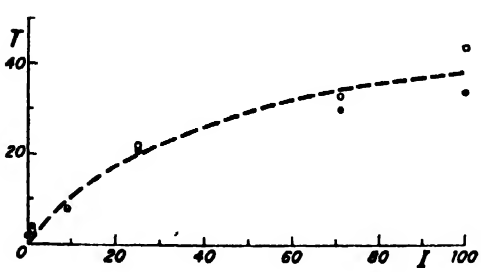

Fig. 1.

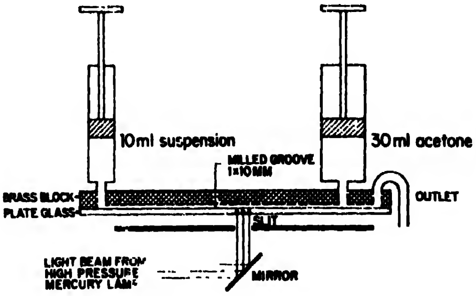

Fig. 2.

for 1/2,000 sec with a photographers flash-lamp. Percent conversion of protochlorophyll is plotted against relative light intensity. It is seen that % conversion increases with increasing light intensity in spite of the fact that the highest intensity is of an order of magnitude 20,000 times full daylight. The curve represents calculated values for a second-order reaction. This reaction goes on without any light-induced loss of pigments.

The next step was to shorten the time between illumination and extraction. Grinding the leaves in a mortar will—compared with time for illumination—take a very long time. I therefore tried to carry out the experiment with chloroplasts suspended in a buffer-solution. Dark-grown wheat plants were disintegrated in a buffer-solution (pH 7.2) containing 0.1 *M* saccharose. After filtration through cotton wool the cell-free filtrate was centrifuged for 10 min in a refrigerated centrifuge at 15,000 *g* and the precipitate was resuspended in fresh buffer-solution. This treatment took place in very faint green light, and the temperature was kept at about 3–4°. Treated in this manner the chloroplasts retain their ability to transform protochlorophyll into

chlorophyll-*a* under illumination, just as in intact leaves. When the chloroplasts are in suspension it is possible to use a flow-method for the conversion-process.

Fig. 2 is a diagram of the experimental arrangement used. By pressing down the pistons simultaneously the chloroplast suspension is forced through a narrow duct. When passing by the slit the photochemical reaction takes place, and when the suspension is mixed with acetone the holochrome is destroyed and the pigments extracted. By keeping the flow-rate constant, the duration of exposure can be varied by means of the slit. The dark-period between illumination and extraction can be varied from zero to a few sec by varying the distance between the slit and the inlet of acetone. The extracted pigments were transferred into ether and measured spectrophotometrically.

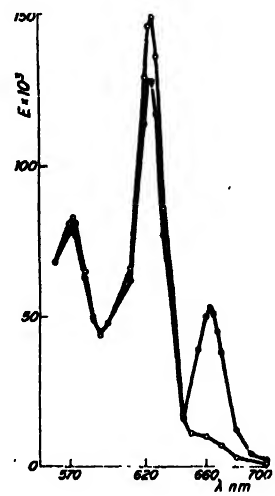

Fig. 3.

Fig. 3 shows the measurements of two samples. Circles represent optical densities of an extract of non-illuminated chloroplasts. There are two peaks on the curve. One at 571 nm and another at 624 nm — this curve is characteristic for protochlorophyll. An identical sample was illuminated in the arrangement shown in Fig. 2. The flow-rate and the slit-width were adjusted to give an exposure-time of 0.04 sec, and there was no dark-period between illumination and extraction. The optical densities of this sample are indicated by dots. There is a decrease at 571 and 624 nm and a new peak at 663 nm representing the formation of chlorophyll-*a*. In this experiment about 20% of the initial amount of protochlorophyll is transformed to chlorophyll-*a* without loss of pigments. I was not able to demonstrate the presence of any dark-reactions by varying the length of the dark-period between illumination and extraction. Here it must be pointed out that the formation of chlorophyll-*a* is measured only as light absorption in ether-extracts.

These experiments give no explanation of the fact that the conversion seems on other evidence to be a second-order process rather than a first-order photochemical reaction. I have therefore tried to formulate another explanation. As it is a well-established fact that only the light that is absorbed by protochlorophyll is effective, we can consider the supply of energy (E_{abs}) to be proportional to the decreasing

concentration of protochlorophyll (c), *i.e.*, $E_{abs} = ck_1$. If we then assume the rate of conversion to be proportional to concentration and proportional to the supply of energy we have $dc/dE = -c^2k_2$. This is the formula for a second-order reaction, and possibly an explanation of the experimental facts.

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Some photochemical properties of phytochrome

The pigment which mediates the effects of red and far-red radiation on plant development has been measured spectrophotometrically in intact plant tissue[1]. Absorption spectra of plant material showed the presence of a pigment which existed in two forms, one absorbing maximally at 660 mμ, the other at 730 mμ. Absorption of light by either form caused its transformation to the other form. The agreement between the absorption spectra and the action spectra of the various red-far-red controlled responses left no doubt as to the functional identity of this pigment. The pigment, which has been named phytochrome, has been extracted from the plant with complete photoreversibility between forms. No indication of a co-factor requirement for this

$$P_{660} \underset{730\ m\mu}{\overset{660\ m\mu}{\rightleftarrows}} P_{730} \qquad (1)$$

reaction has been found. Phytochrome has been detected by its reversible photoreaction in a wide variety of plant tissues. Young, dark-grown seedling plants appear to be the richest sources. It can be extracted in alkaline buffer, and partially purified by ammonium sulfate precipitation at 33% saturation. Phytochrome is a soluble protein which stays in solution when centrifuged at 173,000 g for : h. A difference spectrum of such a solution (Fig. 1) demonstrates the photoreversible reaction of eqn. 1. This was obtained using a commercial double-beam recording spectrophotometer by irradiating

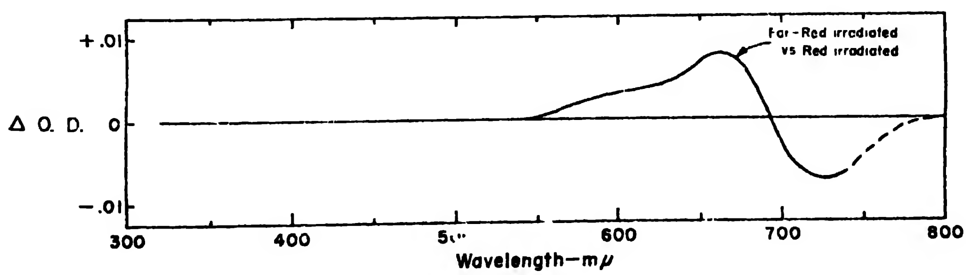

Fig. 1.

the 1 cm sample in the measuring beam with far-red light, and that in the reference beam with red light. The subsequent irradiation of either the sample cuvette with red or the reference cuvette with far-red restored the difference spectrum to the base line.

Reference p. 571

No differences are detectable from 320 to 550 mμ. The difference spectrum at longer wavelengths agrees well with the difference spectrum obtained *in vivo*[1].

A more convenient instrument for detecting phytochrome is the bichromatic difference spectrophotometer, which measures the optical density differences between two fixed wavelenghts selected by interference filters. In the usual assay, wavelenghts of 650 and 730 mμ are used. The intensity of the beams is reduced to prevent them from energising the reaction. The optical density difference between 650 and 730 mμ is measured after irradiation with a red actinic source ($600 < \lambda < 700$) and again after irradiation with a far-red source ($\lambda > 720$ mμ). The change in the optical density difference (referred to as Δ (Δ OD)) is a measure of the sum of P_{660} and P_{730}.

The two forms can be studied independently of one another by setting the bichromatic spectrophotometer to measure the optical density difference between 650 and 800 mμ, or 730 and 800 mμ. The full intensity of the monochromatic beams at 650 and 730 mμ is sufficient to energise the photoreaction. This permits the spectrophotometer to follow the kinetics of the photoconversion as it occurs. Fig. 2 shows the time

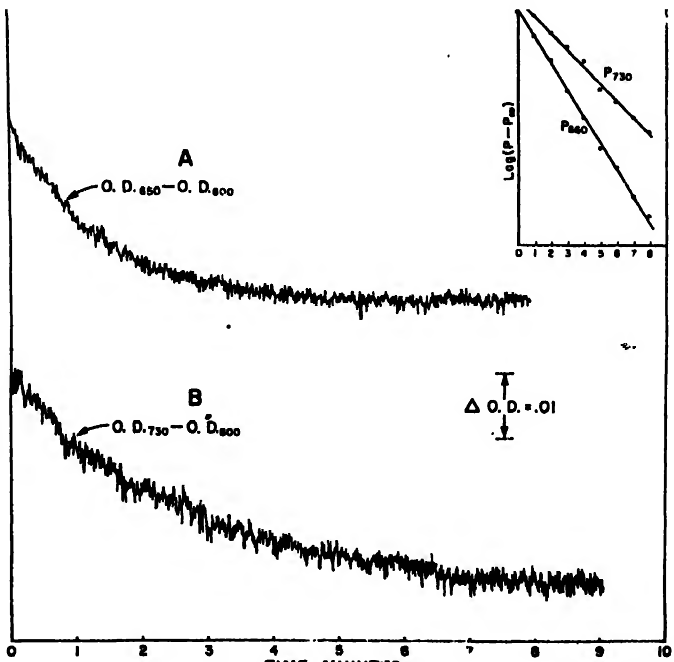

Fig. 2.

course of the optical density difference between 650 and 800 mμ as the spectrophotometer irradiates a clear solution of phytochrome. The solution had been irradiated with far-red light initially to put the phytochrome entirely in the P_{660} form. The similar curve for the optical density difference between 730 and 800 mμ when the solution started in the P_{730} form is also shown in Fig. 2. The semi-logarithmic plots in the insert establish first-order kinetics for both directions of the reversible photoreaction.

The rate equation for the reaction of eqn. 1 under monochromatic illumination is:

$$2.3 \frac{dP_{660}}{dt} = -E_{\lambda}\, \varepsilon_{660_{\lambda}}\, \varphi_{660_{\lambda}}\, P_{660} + E_{\lambda}\, \varepsilon_{730_{\lambda}}\, \varphi_{730_{\lambda}}\, P_{730} \tag{2}$$

This integrates to,

$$\log\,(P_{660} - P_{660\infty}) = -\frac{E_\lambda\,\varepsilon_{660\lambda}\,\varphi_{660\lambda}}{P_{730\infty}}\,t + C \tag{3}$$

E_λ	monochromatic radiation of energy E at λ (Einstein/cm²/sec.)
P_{660}	mole-fraction present at time t
$P_{660\infty}$	mole fraction at $t = \infty$ (same for $P_{730\,\infty}$)
$\varepsilon_{660\lambda}$	extinction coefficient at P_{660} at λ (cm²/mole to the base 10)
$\varphi_{660\lambda}$	quantum yield of $P_{660} \rightarrow P_{730}$ at λ (moles/Einstein)
C	constant of integration $= \log\,(P_{660} - P_{660\infty})$ at $t = 0$

A similar equation can be written for P_{730}. The product of the extinction coefficient and the equation yield can be determined for both P_{660} and P_{730} at any wavelength from the first-order rate constant K,

$$K_{660} = \frac{E_\lambda\,\varepsilon_{660\lambda}\,\varphi_{660\lambda}}{P_{730\infty}}, \quad K_{730} = \frac{E_\lambda\,\varepsilon_{730\lambda}\,\varphi_{730\lambda}}{P_{660\infty}}$$

if the energy of monochromatic radiation and the steady-state mixture are known. A clear solution of phytochrome was irradiated with a uniform field of monochromatic light of known energy from a large spectrograph. At 660 mμ ($\varepsilon_{660} \gg \varepsilon_{730}$ and $P_{730\infty}$ = [illegible] $\cdot\ 10^{10}$ Einsteins/cm²/sec converted half the P_{660} in 19 sec. Calculation shows that:

$$\varepsilon_{660}\,\varphi_{660} = 2.2 \times 10^{7}\ (\lambda - 660\ \text{m}\mu)$$

similarly a measurement at 730 mμ established that:

$$\varepsilon_{730}\,\varphi_{730} = 0.55 \times 10^{7}\ (\lambda = 730\ \text{m}\mu)$$

The optical density measurements show the extinction coefficients of P_{660} and P_{730} are approximately equal at their peaks, therefore

$$\varphi_{660} = 4\ \varphi_{730}$$

Since the quantum yield must be less than or equal to unity a minimum value of the extinction coefficient can be calculated. Expressed in terms of the molar extinction coefficient, α

$$\alpha_{\max} = \frac{1}{1000}\,\varepsilon_{\max} = 2.2 \times 10^{4}\ \text{liter/mole/cm}$$

for both P_{660} and P_{730}.

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[1] W. L. BUTLER, K. H. NORRIS, H. W. SIEGELMAN AND S. B. HENDRICKS, *Proc. Natl. Acad. Sci. U.S.*, 45 (1959) 1703.

The iridophores of the sea urchin *Diadema antillarum* Philippi

The tropical sea urchin *Diadema* is remarkably sensitive to light. The receptors lie in the nervous system, much of which spreads in the skin immediately below the surface epithelium. Consequently, the whole surface is photosensitive. To a degree it resembles an extensive retina, in having not only receptors but also a nervous layer in which interaction between excitation and inhibition may occur[1]. The parallel may be extended further in that extensive movements of screening pigment take place, producing changes not only in colour but also in sensitivity, and there are elements in the skin which recall a tapetum serving to reflect light over the surface. The light reflected is blue and the animal is most sensitive between 455 and 460 mμ.

These reflecting elements, or iridophores, are our main concern here and, appearing as bright blue spots, they contribute significantly to the striking colour and colour change. The skin embodies black and red chromatophores containing respectively a melanin and naphthaquinone pigment resembling echinochrome A. In the darkness the melanin concentrates, leaving a white pattern in the form of a ring around the periproct from which in each inter-radius a white line passes peripherally to bifurcate near the ambitus. When illuminated, at first the edges of the white lines reflect a brilliant bluish lilac, due to the iridophores arrayed along the margins. Later, as the melanophores disperse their pigment beneath the iridophores, the purity of the blue increases and eventually the white areas are obliterated.

The blue spots have been described as eyes in the allied species *D. setosum* and this view has been widely accepted. There is no evidence for this, and some years ago I showed that in *D. antillarum* these structures were not eyes but iridophores[2].

The structure of these organs in *D. setosum* has been described by Sarasin and Sarasin[3], who interpreted their features as those of complex photoreceptors with cornea, lens, retina, etc. Mortensen[4] seems to have been confused, accepting the view that they were eyes, describing their contents as fibrillae wound up like a ball, but figuring them as a hyaline mass!

By micro-dissection we find them to consist of a tough fibrous capsule containing a gel arranged as laminae folded in a variety of ways. The contents can be expelled en masse, or as globules which "set" in sea water to form layers that eventually disintegrate into rod-like bodies, leaving behind in the capsule what appears to be a mass of fibrils. The expelled gel reflects a brilliant blue light. There are no signs of the crystals so often associated with iridophores.

Fixed preparations show a bewildering variety of appearances. After Champy, which preserves most faithfully, the contents of the iridophores are seen to form plates about 1.0 μ wide, consisting of a hyaline core about 0.3 μ across enclosed by a sheath of about the same thickness. The plates extend across the iridophore, but they are folded in various ways and fitted together to form elaborate systems.

The cause of the existing confusion becomes evident when the effect of other fixatives is studied, for they cause the plates to swell and their sheaths disintegrate to form a tangled mass recalling the condition described by Mortensen, so that the fibrous

mass he mentions appears to be the disorganised remains of the sheaths. Further action of fixatives dissolves the remains of the sheaths, leaving the hyaline contents which Mortensen figured.

Their colour is not produced by pigment, because the iridophores do not appreciably absorb light between 550 and 700 mμ. Neither is it produced by fluorescence, for they are invisible in ultra-violet light. Refraction is not the cause, for the colour does not change with the angle of the incident light. Despite the suggestively regular arrangement of the plates, interference or diffraction does not seem to be significantly involved because altering the spacing of the plates by stretching or compression does not alter the colour, neither does rotating the incident beam through angles as great as 35°.

Scattering seems to be the most likely source of colour. The obvious preponderance and the intensity of the reflected blue, together with the slightly yellowish appearance of iridophores viewed by transmitted light and the fact that light reflected from them is polarised, supports this view. The intensity of the blueness in such small structures suggests Rayleigh scattering.

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[1] N. MILLOTT AND M. YOSHIDA, *J. Exptl. Biol.*, 37 (1960) 376
[2] N. MILLOTT, *Nature*, 171 (1953) 973
[3] P. B. SARASIN AND C. F. SARASIN, *Ergeb. Naturw. Forsch. auf Ceylon*, 1 (1887) 1, Wiesbaden.
[4] T. MORTENSEN, *A Monograph of the Echinoidea*, III, 1, C. A. Reitzel, Copenhagen, 1940.

An action spectrum for the inactivation of infectious nucleic acid from tobacco mosaic virus by ultraviolet light**

The action spectra for a number of biological changes induced by ultraviolet light implicate the nucleic acids as the primary absorbers of ultraviolet light. It therefore seemed desirable to see if the action spectrum for the inactivation of a biologically active nucleic acid (RNA) parallels the absorption spectrum. RNA from tobacco mosaic virus (TMV) is easily available in a highly purified state. It exhibits the phenomenon of partial photoreactivation; in the following only the quantum yield data for the inactivation without photoreactivation will be utilized[1]. The comparison we wish to make is outlined as follows: The fundamental equation for inactivation of RNA is[1]

$$\Phi = \frac{C_0 \ln C_0/C}{I_{abs}\, t} \tag{1}$$

where Φ is the quantum yield, C_0 is the initial concentration, and C is the concentration of active RNA after an irradiation time t with an intensity of light I_0. The absorbed intensity, for a path length of one centimeter of solution is I_{abs}. If the solution is very dilute, we may write

$$\Phi = \frac{C_0 \ln C_0/C}{I_0\, \varepsilon\, C_0\, t} = \frac{2.3 \log C_0/C}{I_0\, \varepsilon\, t} \tag{2}$$

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References p. 575

where ε is the molecular extinction coefficient of RNA. Equation (2) is the well known equation of Warburg and Negelein. The extinction coefficient for unit path length is also given by

$$\varepsilon = \frac{\log I_0/I}{C_0} \tag{3}$$

Equating (2) and (3) we obtain

$$\log C_0/C = \frac{I_0 \Phi t}{2.3\, C_0} \log I_0/I = \frac{E_0 \Phi \lambda}{2.3\, C_0\, Nhc} \log I_0/I \tag{4}$$

where E_0 is the incident energy at wavelength λ and N, h, and c are Avogadros number, Planck's constant, and the velocity of light respectively.

Let us call $\log C_0/C$ the *action*, A. The term $\log I_0/I$ is the usual absorbance or optical density, D. Clearly A is proportional to D if the quantum yield is independent of wavelength. In practice it is sometimes easier to measure the action at higher concentrations than permitted by the limiting condition of equation (2), *i.e.*, with I_{abs} greater than about ten percent of I_0. We can then calculate Φ from equation (1) and if Φ is independent of concentration, equation (4) can be used to compute an action for an optically dilute solution at each wavelength for which Φ and D are both known.

Absorbance data for RNA[2] and quantum efficiencies for inactivation[1] are combined to give the action spectrum in Fig. 1. Unfortunately for the comparison, there does seem to be a concentration dependence of the quantum yield[1], but we have assumed that this dependence is independent of wavelength; there is no reason to suspect the contrary. As may be seen, the action spectrum and the absorption spectrum of RNA are in good agreement.

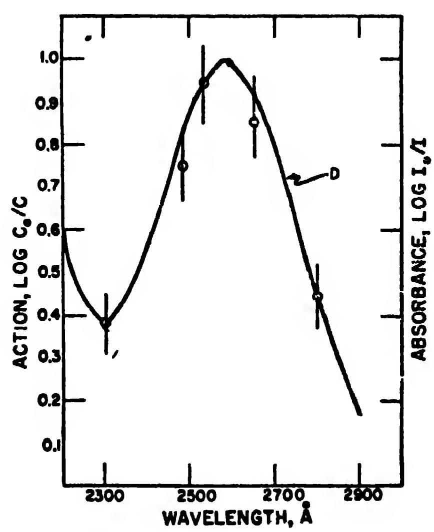

Fig. 1. Action and absorption spectra of infectious nucleic acid (RNA) from tobacco mosaic virus.

We now come to the question of the influence of protein on the action spectrum of RNA. This may be studied by irradiation of the intact virus. Quantum yield calculations are complicated by the necessity of correcting apparent light absorption by the

virus for light scattered by the virus. Also, in dilute solution readsorption of scattered light by the virus will be small, whereas in concentrated solutions, reabsorption may be appreciable. Neglecting the reabsorption, for which we have no means of evaluation, we can correct absorbances for scattering by means of the experimental scattering determinations of McLaren and Takahashi[2] and compute quantum yields for the inactivation of TMV from the rate data of Rushizky *et al.*[1]. As may be seen from Table I, quantum yields for TMV are not independent of wavelength and therefore

TABLE I

QUANTUM YIELDS FOR THE INACTIVATION OF TOBACCO MOSAIC VIRUS, Φ, AND FOR THE RNA INCORPORATED IN THE VIRUS, Φ_s

Wavelength, A	*Rate constants for inactivation* $\cdot 10^6$	$\Phi \cdot 10^5$ *(virus)*	$\Phi_s \cdot 10^5$ *(RNA in virus)*
2804	1.8	2.3	9.3
2652	3.9	4.9	11
2537	4.3	5.8	12
2483	4.0	5.9	14
2300	8.7	~12	~200

the action spectrum can not parallel the absorption spectrum. To find the quantum yields, Φ_s for RNA incorporated in the virus particle, assuming that only light absorbed by the RNA moiety leads to inactivation, Φ must be corrected further for the fraction of the absorbed light absorbed by RNA. These values are also tabulated. It becomes clear that from 2483–2804 Å the sensitivity of RNA in the virus is fairly independent of wavelength, although much less than that of the free RNA (devoid of protein). The quantum yield for free RNA varies from $3.4 \cdot 10^{-3}$–$3.8 \cdot 10^{-3}$ in the range between 2804 and 2300 Å; that is, it is essentially constant within the experimental variability of the tests. On the other hand, Φ_s is much larger at 2300 Å than in the range of 2483–2804 Å, and we suspect that the quantum yield calculated in this way is without physical meaning at 2300 Å. In other words, since Φ for free RNA is independent of wavelength (in the wavelength range under consideration in this paper), we can conclude that the high value of Φ at 2300 Å really means that the virus is rendered non-viable because light absorbed by both the protein and the RNA moieties can lead to inactivation (at 2300 Å most of the light is absorbed by protein). The host plant can not extract active RNA from a coat of protein which has become denatured by U.V.-light, and therefore the quantum yield is higher than would be expected if the RNA was equally available to the plant after irradiation at any wavelength. This conclusion was reached in another way by Siegel and Norman[3].

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1 G. W. Rushizky, C. A. Knight and A. D. McLaren, *Virology*, 12 (1960) 32.
2 A. D. McLaren and W. N. Takahashi, *Biochim. Biophys. Acta*, 32 (1959) 555.
3 A. Siegel and A. Norman, *Virology*, 6 (1958) 725.

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Isolation and function of several newly discovered water-soluble pigments from leaves*

The presence of colored components other than the plastid pigments in breis prepared from leaves has always been apparent. Such yellow, green or brown substances have usually been considered to be decomposition products resulting from the disruption of cells and there has been no systematic attempt to isolate or identify them. In the case of the pigments described in this paper, a blue-green fraction was found in preparations of the protochlorophyll holochrome from dark-grown mutant seedlings of *Helianthus annuus L.*[1]. This water-soluble pigment was present in quantities too large and had properties too unusual to be ignored: In alkaline solution it is green to blue green; it can be reduced by ascorbic acid, hydrosulfite, borohydride, etc. with an accompanying color change to yellow. It is easily reoxidized by shaking in contact with air or by addition of hydrogen peroxide and on acidification, there is a color change to red (the acid-base reaction is also completely reversible but prolonged acidification results in precipitate formation). At all stages of purification, this pigment appears to be bound to protein. Provisionally it has been named allagochrome.

In the course of attempts to purify the crude preparations of allagochrome by electrophoresis, several additional water-soluble fractions were isolated: two yellow pigments (one of which has a yellow-green fluorescence when excited with U.V. light) and a blue fraction. These pigments have been separated not only from chlorophyll-deficient sunflower leaves but also from normal *Helianthus* leaves. A similar series of pigments has been extracted from *Helianthus* seeds. The occurrence of allagochrome (and the accompanying yellow fractions) is not restricted to *Helianthus* and its mutants but has also been found in chard, skunk cabbage and carrot leaves. A systematic survey will probably demonstrate its presence in many other plant species.

METHODS OF PREPARATION

Leaves were ground in glycine-NaOH buffer (pH 9.5, 30 ml buffer for each 10 g leaves) and the resulting brei filtered through several layers of cloth. The filtrate was centrifuged for 1 h at 10,000 g (0°) and the precipitate from this centrifugation (which contained chloroplasts, starch grains and other cellular debris) was discarded. Acetone was added to the supernatant to 25% by volume and this mixture was centrifuged for 15 min at 10,000 g (0°). Once again the precipitate was discarded and additional acetone was added to the supernatant until the solution became cloudy and a precipitate began to form. This precipitate contained allagochrome and the associated pigments and was separated from the suspending solution by centrifugation. Traces of chlorophyll and carotenoid impurities were removed from the precipitate by washing with acetone. The precipitate was dissolved in buffer and further purified by continuous-flow electrophoresis using the glycine–NaOH buffer (50 ml 0.2 M glycine + 22.4 ml 0.2 M NaOH in a total volume of 200 ml) as electrolyte and an applied potential of 450 to 500 V. The fractions obtained from electrophoretic purification, in order of their appearance from left to right across the rack of collecting tubes, were:

* The work reported in this paper was supported by a grant from the National Science Foundation (NSF-G8972).

a colorless fraction (exhibiting a blue fluorescence when excited by U.V. light), a yellow fraction (with a yellow-green fluoresence iu U.V. light), a green fraction blending into a blue-green fraction (or a separate blue fraction in preparations from *Helianthus* seeds) and a non-fluorescent yellow fraction.

The acid form of allagochrome can be transferred into butanol and later recovered with a dilute solution of NH_4OH or alkaline buffer. For a time this technique was used as a step in purification[1]. However, transfer through butanol caused appreciable changes in both the absorption spectrum and the stability of this pigment and this method is therefore no longer used.

NATURE OF THE PIGMENTS

Although the chemical characterization of these pigments is far from complete, it is possible to make some statements about their nature based on preliminary findings. It is quite certain that allagochrome and the associated yellow pigments are protein complexes because they have many properties commonly associated with proteins, *e.g.*, electrophoretic migration, high optical densities in the ultraviolet (see Figs. 1, 3 and 4), precipitation by dilute solutions of $BaCl_2$, $HgCl_2$ and dilute mineral acids, by concentrations of acetone greater than 50%, and by $(NH_4)_2SO_4$ in concentrations greater than 50% saturation.

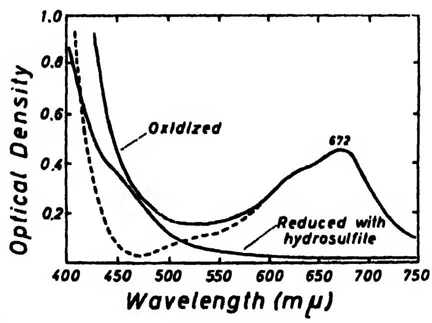

Fig. 1. Absorption spectrum of a crude preparation of allagochrome from *Helianthus* leaves. Spectra of both oxidized and reduced forms are shown. The broken line is the spectrum of the oxidized form measured against the reduced form as solvent blank.

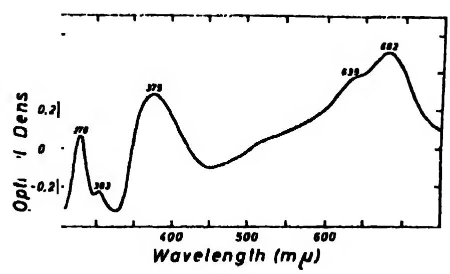

Fig. 2 Absorption spectrum of electrophoretically pure allagochrome from *Helianthus* seeds measured against pigment reduced with borohydride as solvent blank

Purified preparations of allagochrome have been digested with concentrated sulfuric and nitric acids. Dithizone derivatives prepared from these digests have absorption spectra characteristic of copper. On the basis of crude estimates of the molecular weight of this pigment by a diffusion technique it now appears that there is one copper atom per molecule of approximately 50,000 molecular weight.

The striking color changes of allagochrome on oxidation or reduction (absorption spectra of the oxidized and reduced forms in the visible portion of the spectrum are shown in Fig. 1) and in response to the pH of the medium indicate that the colored moiety of the pigment–protein complex may be a quinone; its red color in acid solution suggests an indole nucleus. The striking similarities between the color changes of allagochrome and those of hallachrome, a naturally occurring quinone isolated from the marine polychaete, *Halla parthenopea*, by Mazza and Stolfi[2] support this hypothesis.

The fluorescent yellow fraction is the only other pigment in this group about which

References p. 580

it is possible at this time to make any prediction concerning chemical structure. I absorption spectrum and the color of its fluorescence suggest that it may be a flav

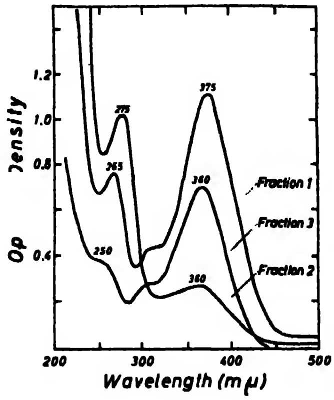

Fig. 3. Absorption spectra of three yellow fractions obtained by continuous-flow electrophoresis from crude extracts of *Helianthus* seeds.

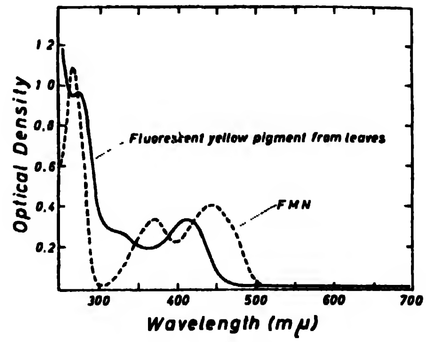

Fig. 4. Comparison of absorption spectra of flavin mononucleotide and yellow fluorescent fraction from *Helianthus leaves*.

protein. Fig. 4 compares the absorption spectrum of an electrophoretically pure preparation of this pigment with that of a dilute solution of flavin mononucleotide.

Although the pigments prepared from *Helianthus* seeds are quite similar to those prepared from leaves, their molecular size appears to be smaller (relatively smaller optical densities in the ultraviolet). Also, two additional non-fluorescent yellow fractions appear on electrophoretic separation. There is a very slow conversion of these two fractions to green and blue-green forms. Fig. 3 shows the absorption spectra of three of the yellow fractions collected during the electrophoretic separation of sunflower seed extracts. The similarity of their absorption spectra to the pattern observed when the spectrum of an oxidized preparation of seed allagochrome is measured against the reduced form of this pigment as a solvent blank should be noted (Fig. 2).

PHYSIOLOGICAL FUNCTIONS

At this time there is experimental evidence to support hypotheses concerning physiological function for only one of the pigments described in this paper, *i.e.*, the green pigment allagochrome. The ease and reversibility of oxidation and reduction of this pigment suggest that it may participate in the hydrogen or electron transfer systems of either respiration or photosynthesis.

Hill and Walker[3] have reported a stimulation of rates of photosynthetic phosphorylation by pyocyanine and they concluded that the previously known activity of phenazine methosulfate in phosphorylating systems is due to its rapid conversion to pyocyanine in the light. Preliminary experiments indicate that allagochrome can activate photosynthetic phosphorylation by spinach chloroplasts. It is possible that this pigment is a naturally occurring counterpart of pyocyanine in phosphorylating systems *in vivo*. Unlike pyocyanine, allagochrome acts only in the presence of oxygen and catalyzes an oxidative or non-cyclic photophosphorylation.

More evidence exists concerning the involvement of allagochrome in the hydrogen transport system of respiration, specifically as a terminal oxidase. Addition of allagochrome to a suspension of aerobically cultured yeast caused some acceleration of

respiratory oxygen uptake (30 to 40%). More significant, however, was the observation that when rates of oxygen uptake had first been inhibited with cyanide, there was

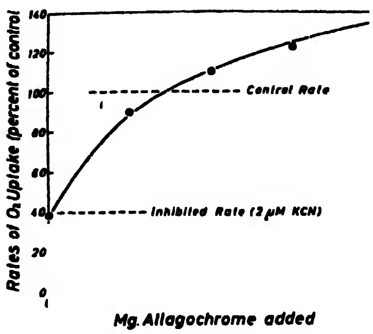

Fig. 5. Relative rates of oxygen uptake after addition of varied amounts of allagochrome to yeast suspensions inhibited with 2 μmoles KCN. Vessel contents: 1 ml yeast suspension (O.D. = 1 at 525 mμ) in 0.5% buffered sucrose ($M/15$ phosphate buffer, pH 7.5), 2 μM KCN, 0 to 8 mg allagochrome, 0.2 ml 10% KOH in center well, total volume: 3 ml. Control rate was – 0.60 μl O_2/min per vessel.

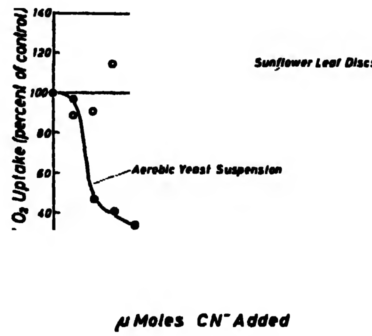

Fig. 6. Comparison of effects of cyanide on rates of respiratory oxygen uptake of yeast and sunflower leaves. Vessel contents (*Helianthus* leaves): leaf discs (0.5 cm in diameter, fresh wt. approx. 200 mg) suspended in water plus 0 to 8 μM KCN, 0.2 ml 10% KOH in center well. Total vol.: 3 ml. Control rate was –0.53 μl O_2 per min per 100 mg fresh wt. Vessel contents (yeast): 3 ml yeast suspension (concentration and suspending medium as for Fig. 5), 0 to 2 μM KCN, 0.2 ml 10% KOH in center well. Total volume: 4 ml. Control rate was –1.96 μl O_2/min per vessel.

a complete reversal of inhibition and even stimulation over control rates if sufficient pigment was added (Fig. 5). It thus appeared that allagochrome could substitute for the cyanide-inhibited cytochrome oxidase. The respiration of sunflower leaves (the source of allagochrome) was found to be exceedingly resistent to cyanide poisoning. Leaf discs suspended in solutions of cyanide up to four times the concentration sufficient to reduce yeast respiration to a level about one-third that of the controls showed no inhibition of oxygen uptake (Fig. 6). It has also been shown spectrophotometrically that allagochrome is able to oxidize reduced cytochrome *c* and that its ability to mediate this oxidation is not inhibited by cyanide. These observations support the hypothesis that allagochrome may be a terminal oxidase of sunflower leaf respiration. It may indeed be the cyanide-resistent terminal oxidase which apparently is present in many plant tissues[4,5]. If this is the case, then one would predict that those plant tissues in which a major portion of respiratory oxygen uptake is insensitive to cyanide should contain allagochrome or a closely related pigment. This prediction certainly holds true for mature carrot leaves which have been found to be an excellent source of allagochrome.

ACKNOWLEDGEMENTS

Experiments on the effects of allagochrome on photosynthetic phosphorylation were done in collaboration with Dr. Albert Krall (R.I.A.S., Baltimore). Manometric measurements of respiration were done by Miss Mary L. Lutz. Her participation in the development of this aspect of the problem is very much appreciated.

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References p. 580

1 HELEN M. HABERMANN, in M. B. ALLEN (ed.), *Symposium on Comparative Biochemistry of Photoreactive Systems*, Academic Press, New York, 1960, p. 73.
2 F. P. MAZZA AND G. STOLFI, *Boll. soc. ital. biol. sperim.*, 5 (1930) 74.
3 R. HILL AND D. A. WALKER, *Plant Physiol.*, 34 (1959) 240.
4 CONRAD S. YOCUM AND DAVID P. HACKETT, *Plant Physiol.*, 32 (1957) 186.
5 DAVID P. HACKETT, *Ann. Rev. Plant Physiol.*, 10 (1959) 113.

DNA content and U.V.-resistance of *Lactobacillus leichmannii*

The main part of the investigation to be communicated here was accomplished together with Dr. A. Wacker[1,2], now in Frankfurt/Main. Wacker *et al.*[3] found that the content of deoxyribonucleic acid (DNA) in 100 mg of dried cells of *Lb. leichmannii* 313

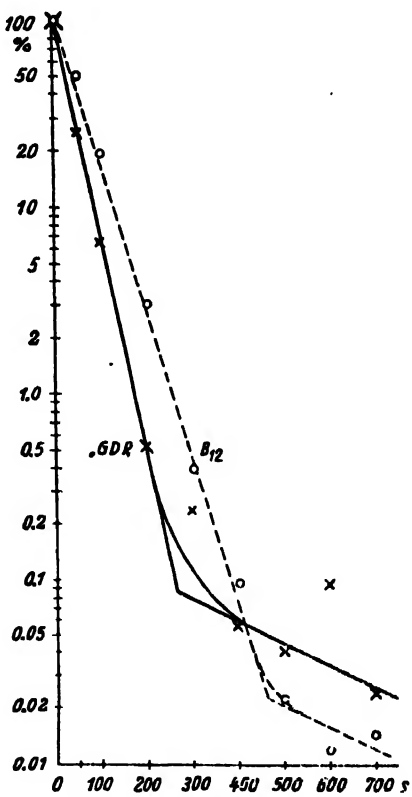

Fig. 1.

decreased from more than 2 mg to less than 1 mg, if the growth substance vitamin B_{12} was replaced by a deoxyriboside in the nutrient medium. The inactivation of a cell by irradiation is now commonly interpreted as a lethal mutation, and the DNA is held to be responsible for bearing the genetical information. This induced us to investigate the U.V.-sensitivity of these cells which are genetically homogenous but whose DNA content varies by a factor of almost 3.

The cultivation of the strain and the composition of the nutrient medium have

already been described[4]. The medium is a solution of acetate buffer with vitamins to which are added either 0.01 µg/ml vitamin B_{12} or 5 µg/ml guaninedeoxyriboside (GDR). Starting from a stab-culture in B_{12}-agar, 3 inoculations were carried out in liquid media containing B_{12} and GDR respectively. After 22 h, the 3 cultures were washed in the centrifuge and then suspended in saline for irradiation. They were irradiated from above vertically using a mercury high pressure lamp giving mainly a wavelength of 254 mµ. For the determination of the survival the bacilli were incubated according to the method of Fortner because of their microaerophily. In this method, one half of the nutrient agar in the Petri-dishes is inoculated with *Serratia marcescens* which consumes the unwanted oxygen in the air-tight closed dishes. Both subcultures of *Lb. leichmannii* (grown with B_{12} and GDR respectively) were inoculated after irradiation onto agar containing B_{12}. After incubation at 37° for 48 h the bacteria had grown to colonies with a diameter of 1–1.5 mm. All operations after the irradiation were carried out in yellow light in order to avoid possible photoreactivation.

Fig. 1 shows the results of 5 experiments. It is distinctly seen that the U.V.-resistance of cells possessing more DNA is higher. Both curves, however, are of single hit type. The factor of dose reduction is equal to 1.4. We see that a purely nutritive physiologically induced difference in the DNA content is followed by a quantitatively different U.V.-resistance of the cells.

Before attempting to interpret these facts it is necessary to ask whether differences in RNA content also exert some influence. Wacker *et al.*[3] have, however, already demonstrated that the cells of *Lb. leichmannii* contain the same amount of RNA whether grown in the presence or in the absence of B_{12}. This finding eliminates RNA from the discussion.

A genetical interpretation of U.V.-inactivation (which was applied *e.g.* by Stein and Laskowski[5–7] with some success to their interesting investigations in races of yeast with differing degrees of multiploidy) can describe the inactivation either as a recessive or as a dominant lethal mutation. For three reasons the increase of DNA in the cells of *Lb. leichmannii* and the increase of U.V.-resistance connected with it cannot be due to polyploidization. (1) It has never been found that the degree of polyploidy is dependent on the nutrition of the cell, and could be altered at will by it. (2) In the case of a recessive lethal mutation as an inactivating event, the polyploid cells must show multiple hit curves. This is not observed (Fig. 1). (3) In the case of dominant lethal mutations the cells of the higher degree of polyploidy should be more sensitive. This is opposite to the observation (Fig. 1).

Wacker *et al.*[8] found that in their attempts to incorporate 5-bromouracil into the DNA of some bacteria, they were not able to replace all thymine, but, for instance in *Enterococcus Stei*, at best 70%. This suggested to them that some bacterial strains might contain two kinds of DNA, a genetical one into which no 5-bromouracil can be incorporated, and a nongenetical one in which thymine can be replaced by 5-bromouracil.

This concept of a reserve-DNA might well help to explain the findings demonstrated here. We have only to suppose that the cells grown with GDR contain either very little or no reserve-DNA. This would mean that the GDR-cells were showing the true U.V.-sensitivity of *Lb. leichmannii*. The cells grown with vitamin B_{12} however, apparently possess some reserve-DNA. The quanta of U.V. absorbed by this reserve-DNA do not disturb the viability of the cell at all. This readily explains the observa-

References p. 582

tion that for the same inactivation, a higher dosis is required for cells with more DNA than in those with less DNA.

At the moment nothing is known about the distribution of these two components of DNA, the assumed reserve-DNA and that with genetical information. Can the reserve-DNA carry the same genetical information as the other, so that at least a part of the genome exists in a polyploid state? This has already been shown to be unlikely since for the explanation mentioned above it is necessary to suppose that the reserve-DNA does not carry genetical information.

This leads to a further consequence. In the light of our present knowledge of the structure of DNA, "informationlessness" probably results from a regular sequence of the side bases involved (thymine, adenine, guanine, cytosine) *e.g.* TATATA . . . But it is very difficult to imagine how this "informationless" reserve-DNA could transform itself or could be transformed by metabolism of the cell into the structure with information, by alteration of the sequence of bases, when the prototype had been damaged or destroyed by absorption of U.V.-quanta. In other words, it is unlikely that the reserve DNA exerts a restoring function -- it is more likely that it acts as a protective substance by absorbing damaging U.V.-quanta.

The situation has become a little more complicated as a result of our latest experiments with photoreactivation (PHTR). We used the whole spectrum of a high pressure mercury lamp filtered through 8 mm window-glass. Immediately after U.V.-irradiation the bacteria on the B_{12}-containing acetate--agar were irradiated for 2 h at a distance of 63 cm.

We found no PHTR at the first steep part of the survival curve for cells grown with GDR (Fig. 1), but a reactivation by an average factor of 2 for cells grown with B_{12} in both parts of the curve. (The reason for PHTR of cells grown with GDR in the flatter part of the survival curve, Fig. 1, cannot be discussed here). We have not yet applied the optimal wavelengths for PHTR of *Lb. leichmannii*. If this were done, one could expect a much greater PHTR than was found in the present work.

The existence of PHTR in cells with a great deal of reserve-DNA and its absence in those with less or without reserve-DNA, tends to support the idea of the restoring function of reserve-DNA, an idea which has been shown to be unlikely above.

Clearly, further experiments are required before the matter can be settled.

The author is greatly indebted to Mr. E. R. Lochmann, Berlin, for supplying him with nutrient media.

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[1] W. ECKART AND A. WACKER, *Z. Naturforsch.*, 15b (1960) 429.
[2] W. ECKART AND A. WACKER, *Monatsber. deut. Akad. Wiss. Berlin*, 2 (1960) 297.
[3] A. WACKER, D. PFAHL AND I. SCHRÖDER, *Z. Naturforsch.*, 12b (1957) 510.
[4] A. WACKER, A. TREBST AND F. WEYGAND, *Z. Naturforsch.*, 11b (1956) 7.
[5] W. STEIN AND W. LASKOWSKI, *Z. Naturforsch.*, 13b (1958) 651.
[6] W. STEIN AND W. LASKOWSKI, *Naturwissenschaften*, 46 (1959) 88.
[7] W. LASKOWSKI AND W. STEIN, *Naturwissenschaften*, 47 (1960) 163.
[8] A. WACKER, A. TREBST, D. JACHERTS AND F. WEYGAND, *Z. Naturforsch.*, 9b (1954) 616.

Photometabolism of benzoic acid by *Athiorhodaceae**

In nonsulfur photosynthetic bacteria, light-dependent carbon dioxide reduction is linked to the oxidation of organic substrates. The substrate can be metabolized either photosynthetically under anaerobic conditions, or heterotrophically in darkness with molecular oxygen serving as the hydrogen acceptor. When these organisms are illuminated in the absence of substrate, measurements of redox potentials provide evidence that oxidizing substances are formed[1,2], but molecular oxygen cannot be detected[3]. The apparent equivalence of light and oxygen for substrate utilization suggests that the oxidant generated by the photochemical reaction can function like molecular oxygen. To test the validity of this suggestion, we can determine if photosynthetic bacteria can oxidize aromatic substrates such as benzoic acid.

Previous investigations[4] have shown that the primary attack on the aromatic nucleus by bacterial enzymes involves the simultaneous introduction of two hydroxyl groups. If peroxidation or some other oxygen-requiring mechanism is the first step in the microbiological cleavage of aromatic carbon–carbon bonds, then the photometabolism of compounds such as benzoic acid under strictly anaerobic conditions may be a useful measure of the oxidizing power generated by the photochemical reaction in bacterial photosynthesis.

To determine if benzoic acid or other aromatic compounds could serve as organic substrates for anaerobic photosynthesis, elective cultures were designed to select for nonsulfur photosynthetic bacteria. In these experiments, the aromatic compound was supplied as the sole substrate in a mineral medium supplemented with trace quantities of B vitamins to satisfy the growth factor requirements of the auxotrophic *Athiorhodaceae*[5]. Glass-stoppered bottles completely filled with the synthetic medium were inoculated with samples of mud and exposed to continuous light at 28–30°. From such elective cultures, several strains of purple bacteria with the characteristics of the genus *Rhodopseudomonas* were isolated. When representative species of *Athiorhodaceae* from the culture collection at Hopkins Marine Station were tested, strains of *R. palustris*, *R. capsulatus*, *R. spheroides*, and *R. gelatinosa* as well as *Rhodospirillum rubrum* were found to grow well anaerobically in the light with benzoate as substrate. Thus the ability to utilize benzoic acid as a substrate during anaerobic photosynthesis appears to be widespread among the *Athiorhodaceae*.

In the following growth and manometric experiments, *Rhodopseudomanas palustris* was used as experimental object. Growth experiments were carried out in illuminated glass-stoppered bottles as described previously. Increase in cell number measured as optical density or dry weight of cells was found to be proportional to the concentration of substrate provided. When acetate was compared with benzoate as a substrate for *R. palustris*, the total yield per mg substrate with benzoate was found to be roughly three times that of acetate (Fig. 1).

* Portions of this work were supported by a postdoctoral fellowship from the American Cancer Society while the author was at Hopkins Marine Station of Standard University, Pacific Grove, California.

References p. 585

Much of the evidence for the intermediary metabolism of substituted aromatic compounds in bacteria has come from the application of the simultaneous adaptation technique[6]. By analogy with non-photosynthetic bacteria, the enzymes operating in the oxidative pathway of aromatic compounds in *Athiorhodaceae* should be inducible. If benzoate is oxidized by way of a dihydroxy intermediate, then benzoate-grown cells should attack catechol or protocatechuic acid without an adaptation period. When oxygen consumption was measured manometrically, in dark experiments,

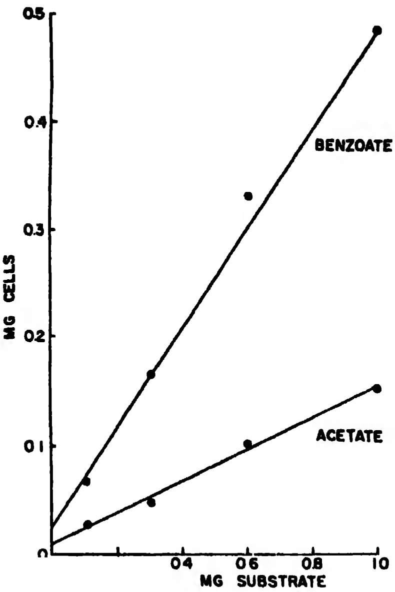

Fig. 1. Total yield of *Rhodopseudomonas palustris* cells as a function of concentration of substrate, anaerobically cultivated in light.

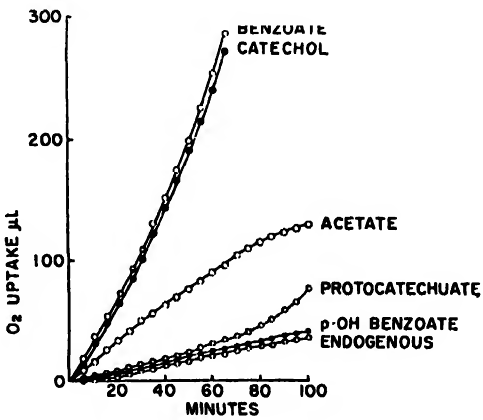

Fig. 2. Dark oxidation of aromatic compounds by benzoate-grown cells of *Rhodopseudomonas palustris*.

resting cells of *R. palustris* that had been adapted to grow on benzoate were found to oxidize benzoate and catechol at the same rate; protocatechuic acid was attacked only after a period of adaptation. In contrast to the dihydroxy compounds, *p*-hydroxybenzoate was not oxidized by benzoate-adapted cells during the course of short term manometric experiments (Fig. 2). It seems logical to conclude that the dihydroxy-substituted compound catechol lies on the oxidation path of benzoate, but that the mono-hydroxy compound does not. We cannot say with certainty that the pathway of benzoate oxidation in *R. palustris* follows the scheme proposed by Stanier[4]. Nevertheless, the apparent involvement of a dihydroxy- rather than a mono-hydroxy-substituted compound as an intermediate argues in favor of a reaction mechanism in which two hydroxyl groups are simultaneously introduced into the benzene nucleus.

On comparative biochemical grounds, the oxidation of organic substrates by a photochemical oxidant is analogous to the non-photosynthetic oxidation of hydrogen donors by an inorganic source of "bound oxygen". Since the reduction of nitrate is essentially the use of "bound oxygen" as hydrogen acceptor, it might be expected that the oxidation of benzoate would proceed under anaerobic conditions in the presence of nitrate as inorganic oxidant. Enrichment experiments for denitrifying organisms utilizing benzoate as hydrogen donor have yielded bacteria that reduce nitrate to nitrogen concomitant with the oxidation of the aromatic hydrogen donor.

The general equation for the microbiological formation of methane shows certain similarities to carbon dioxide reduction reactions in photosynthesis and suggests that carbonate may serve as an hydrogen acceptor for the anaerobic oxidation of aromatic compounds such as benzoate. In enrichment cultures from sewage sludge, high yields of methane were obtained with benzoate as substrate[7].

By analogy with nitrate and carbonate, one can envisage an anaerobic oxidation of aromatic substrates by organisms utilizing sulfate as an ultimate hydrogen acceptor; however, elective cultures for sulfate-reducing micro-organisms with such capabilities have thus far been negative (see *Note added in Proof*).

In essence, the utilization of benzoic acid by photosynthetic bacteria under anaerobic conditions seems to parallel the anaerobic oxidations dependent upon a bound form of oxygen as hydrogen acceptor. This suggests that the oxidant generated by the photochemical reaction may be a bound form of oxygen of sufficient positive potential to function in reactions normally requiring molecular oxygen as obligatory oxidant. Like nitrate, the photochemical oxidant may require the participation of a metallo-enzyme of the oxidase type in order to function as hydrogen acceptor. It is also reasonable to assume that in the absence of inorganic oxidants such as nitrate, the photochemical oxidant is derived from water.

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1 P. A. ROELOFSEN, *On the Photosynthesis of the Thiorhodaceae*, Dissertation, Utrecht, 1935.
2 E. C. WASSINK, *Antonie van Leeuwenhoek, J. Microbiol. Serol.*, 12 (1947) 281.
3 H. MOLISCH, *Die Purpurbakterien nach neuen Untersuchungen*, Fischer, Jena, 1907.
4 R. Y. STANIER, *Bacteriol. Rev.*, 14 (1950) 179.
5 S. H. HUTNER, *J. Bacteriol.*, 52 (1946) 213.
6 R. Y. STANIER, *J. Bacteriol.*, 54 (1947) 339
7 F. M. CLARK AND L. R. FINA, *Arch. Biochem. Biophys.*, 36 (1952) 26.

Note added in proof: Recent experiments in collaboration with Dr. Kjell Eimhjellen at the Institute of Biochemistry, Norwegian Technical University, Trondheim, have shown that *o*-phthalic acid can serve as sole carbon source for sulfate-reducing bacteria. Cultures of *Desulfovibrio* isolated from soil at Trondheim metabolize phthalate under strictly anaerobic conditions with sulfate as inorganic oxidant. Analysis of *Desulfovibrio* cultures grown on phthalate indicate that the reduction of sulfate to sulfide is accompanied by an incomplete oxidation of phthalic to acetic acid. If the metabolism of phthalic acid by sulfate-reducing bacteria proceeds *via* a dihydroxy intermediate as in the soil bacteria reported by Ribbons and Evans (*Biochem. J.*, 76 (1960) 310), then it would provide additional comparative biochemical support of the postulate that the photochemical oxidant corresponds in function to the inorganic hydrogen acceptor of non-photosynthetic anaerobes.

Kinetics of a stepwise photoxidation of ascorbic acid by a manganese-flavin-catalase system

The photochemistry of chloroplast preparations has become an extensive branch of research in photosynthesis. As more substrates, activators, catalysts and co-enzymes are added to reaction mixtures containing chloroplasts, it becomes increasingly difficult to interpret correctly the reactions in such mixtures under the influence of light. Any explanation would become simpler if similar reactions could be observed under conditions where some of the more complex constituents of the mixture could be omitted --- for instance the chloroplasts themselves. Flavin, ascorbic acid, manganous ion and heme-protein are some of the substances which have been assigned special roles in the photochemistry of chloroplasts; flavin and ascorbic acid in photophosphorylation, manganese and cytochromes in the photochemical evolution of oxygen. Free oxygen on the other hand is known to be reduced to hydrogen peroxide (and

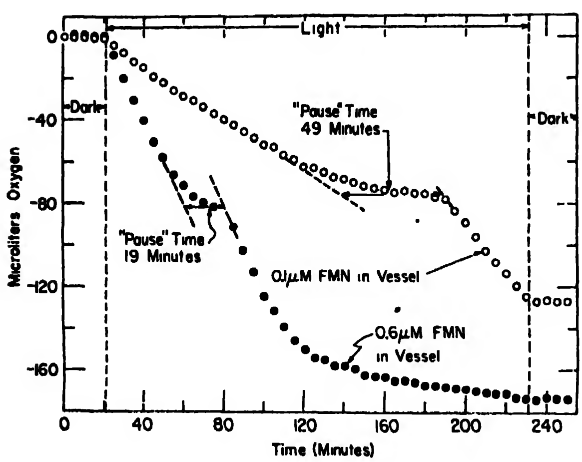

Fig. 1. Representative time courses of the three steps of photoxidation of ascorbic acid at low and high FMN concentrations. A higher concentration shortens the time needed for completion of each step. Note the arbitrary determination of "pause" time as time between linear rates of oxygen uptake in the first and third steps. Vessel contents: 0.1 or 0.6 micromoles FMN, 4 micromoles ascorbic acid, 4 mg catalase, 0.1 cc. 50% ethanol, 4 micromoles Mn^{++}, buffer to 4 ml.

eventually water) by illuminated chloroplasts[1,2] and such oxygen consumption leads, in the presence of flavin mononucleotide and hem[illegible] [illegible], to a much enhanced rate of phosphorylation[3]. A look at the recent [illegible] chloroplast reactions will easily convince the reader of the complexity [illegible] and the difficulty of explaining them in an orderly manner.

In the present paper we describe and discuss the reaction which starts the moment a buffered solution containing [illegible]in, ascorbic acid, manganous ions, catalase and alcohol is illuminated. The alcohol serves to eliminate hydrogen peroxide but otherwise is unimportant.

The presence of only one catalytic heme-protein (catalase, or cytochrome, or hem[illegible]globin — in diminishing order of activity) and quite specifically of manganous i[illegible] (no other metal ion tested was active) is responsible for a photoxidation of ascor[illegible] acid which differs markedly from those previously described. Fig. 1 shows the photo[illegible]dation of ascorbic acid as the time course of oxygen uptake during a manomet[illegible]

experiment. We can distinguish at least three different phases which neatly follow one another.

The photosensitivity of flavins has been known since the time they were first isolated. A photoxidation of ascorbic acid by flavins to the level of dehydroascorbic acid was described long ago[4-6]. What is new in our experiments is the sudden uptake of a second equivalent of oxygen under continued illumination provided that manganous ions and catalase are present. The two oxidative steps are separated by an intermediate reaction, which, under our conditions, is also light dependent and runs to completion before the uptake of the second equivalent of oxygen begins. The third step, *i.e.* the second oxygen-consuming step, is the oxidation of dehydroascorbic acid. All reactions and rates typical for this third phase in the curves of Fig. 1 could be duplicated when dehydroascorbic acid was used as the initial substrate. What the intermediate step, the "pause" without a visible gas exchange, consists of, we do not know. In many experiments of the same type it was never shorter than ten minutes. One of the final oxidation products was identified as glyoxylic acid. The other may (but we are not yet certain) be 2-keto-dihydroxy-butyric acid. Each of the successive photochemical reactions appears in turn to consist of more than one reaction step.

The rate in the dark of autoxidation of the complete system was negligible and remained so during illumination with red light which is not absorbed by flavin. If, however, in addition to the substances enumerated above, fresh chloroplasts were introduced into the solution, the reaction took its course in red light in the typical three step sequence. This means that the chlorophyll in the grana can somehow be coupled to this completely artificial system so that red light becomes effective.

Obviously we have to ask: How is it possible that in an homogeneous solution a reaction whose substrate is the product of two earlier transformations proceeds faster than any one of the preceding reactions, yet will not begin until these transformations have been practically completed? What causes at least three types of molecules, each of them capable of some reaction induced by the same sensitizer (the FMN molecules), to wait in line until their turn comes, instead of competing freely among one another according to the mass action laws of chemistry?

There are several possible hypotheses. We prefer a rather simple one and assume *that on the molecular level* the solution is not homogeneous. Part of the reacting molecules are not freely dissolved but form *associations or complexes strong enough to determine an order of succession on the basis of specific affinities.*

It just happens that our starting material, ascorbic acid (or an early intermediate), associates more strongly than its oxidation products with that reactive flavin form which is uniquely responsible for any further photochemical transformation. This kind of selection must occur at least twice. Since catalase and manganous ion and flavin (or FMN) together are indispensable for the completion of the reaction, let us assume that these three produce a new molecule, a photocatalyst with quite specific properties. It is important that of all the substances in our solution, catalase, with a molecular weight of 250,000, is present in the lowest molar concentration, about an order of magnitude lower at least than any of the other components. Thus we have a quite small but very effective fraction of bound FMN and an excess of it in the medium. This is the conclusion we have to draw from Fig. 2 where the rate of oxygen uptake by ascorbic acid and dehydroascorbic acid is plotted against the increasing concentration

References p. 588

of FMN. The lower curve saturates at about 10^{-4} *M* FMN. Any light absorbed by an excess of flavin in solution is of no use for the last part of the reaction. But it can contribute, though less effectively, to the photoxidation of the initial substrate ascorbic acid. The bend in the rate *versus* pigment concentration curve for ascorbic acid (upper curve) comes at the same FMN concentration.

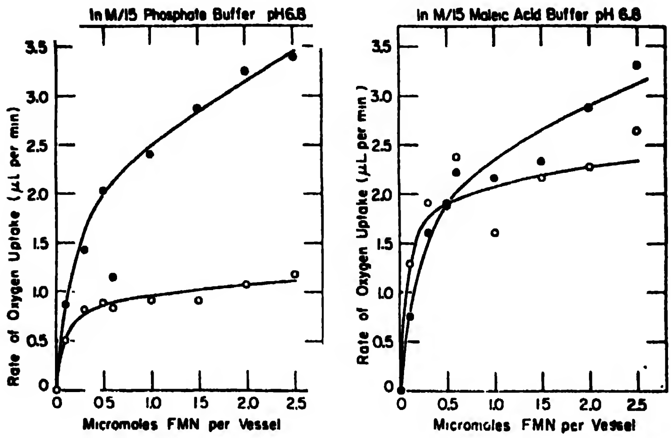

Fig. 2. Dependence of rate of photoxidation on FMN concentration, and on nature of buffering medium. Rates of photoxidation of ascorbic acid ●——●. Rates of photoxidation of dehydroascorbic acid O - O. Left: Reactions run in $M/15$ phosphate buffer, pH 6.8. Right: Reactions run in $M/15$ maleic buffer, pH 6.8. Vessel contents: 0 to 2.5 micromoles FMN, 4 micromoles ascorbic acid, 4 mg catalase, 0.1 ml 50% ethanol, 4 micromoles Mn^{++}, buffer to 4 ml.

It follows that ascorbic acid can be photoxidized by both forms, but its oxidation products only by the catalase complex. In our experiments the reactive centers of the catalase molecules become fully active and saturated when the concentration of flavin and $MnCl_2$ reaches about 10^{-4} *M* in the solution.

The protein-catalyzed oxidation of ascorbic acid is the faster reaction of the two but it is limited by the relatively small number of catalase molecules in the vessel. At higher light intensities and higher pigment concentrations the photoxidation in solution may easily produce the larger effect. But unless the bulk of the ascorbic acid has been oxidized first, the oxidation products have little chance to find a free place at the catalytic centers. A true enzyme-like specificity finally appears with the third oxidation step which requires manganese. Thus we may say that we have here a synthetic photo-metallo-flavo-protein oxidase for dehydroascorbic acid with a surprising specificity in the combination of manganous ion with a heme-protein. How truly specific it is for the substrate we have not yet tested. The ease with which it can be coupled to the action of light-excited chloroplasts, or specifically inhibited by the action of copper ions, gives the reaction a biological interest besides that of being a photochemical process with rather odd reaction kinetics.

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[1] A. H. MEHLER, *Arch. Biochem. Biophys.*, 33 (1951) 65.
[2] H. M. HABERMANN, *Plant Physiol.*, 35 (1960) 307.
[3] T. NAKAMOTO, D. W. KROGMANN AND B. VENNESLAND, *J. Biol. Chem.*, 234 (1959) 2783.
[4] L. MARTINI, *Boll. soc. ital. biol. sper.*, 9 (1934) 1235.
[5] F. G. HOPKINS, *Compt. rend. trav. lab. Carlsberg*, 22 (1938) 226.
[6] DAVID B. HAND, E. G. GUTHRIE AND P. F. SHARP, *Science*, 87 (1938) 439.

Session 7

PHOTOCHEMISTRY

Chairman: CYRILLE SIRONVAL, Gorsem (Belgium)

Secretary: AAGE KJER, Copenhagen (Denmark)

Untersuchungen über Verfärbungen von Mepacrin-, Chloroquin- und Phenothiazinlösungen durch UV-Strahlen*

In Arbeiten von Wels und Mitarbeitern[1,2] ist die Bedeutung der reduzierenden Gruppen der Haut für die medizinischen Lichtwirkungen hervorgehoben worden. In erster Linie handelt es sich dabei um fixe Sulfhydrilgruppen (Thiole) und Ascorbinsäure. Sie reduzieren, wenn UV-Bestrahlung erfolgt, Metall- und Metalloidverbindungen in verstärktem Masse, nämlich bis zum Metall oder Metalloid und bringen sie in dieser Form in der Haut zur Ablagerung. Umwandlungen organischer Substanzen (Nitroverbindungen) beruhen dagegen nicht in jedem Falle auf Reduktion; denn als ausgesprochene Redoxsysteme können Sulfhydrilkörper und Ascorbinsäure mit organischen Verbindungen auch auf andere Weise in Reaktion treten, zumal bei Bestrahlung mit UV-Licht.

Um derartige Vorgänge zu studieren, wurden die Arzneimittel Mepacrin, Chloroquin und Phenothiazin** untersucht. Den Lösungen oder Suspensionen dieser Stoffe wurden 0.5% Cystein oder entsprechende Mengen Glutathion oder 0.5% Ascorbinsäure oder 10% Glukose zugesetzt. Nach Einstellung auf ein pH von 7.0 wurden sie bei Wasserkühlung und ständiger Durchmischung bis zu mehreren Stunden der Bestrahlung mit UV-Licht ausgesetzt, wobei die Entfernung der Lichtquelle vom Bestrahlungsobjekt etwa 20 cm betrug. Zum Vergleich wurden Lösungen ohne Zusatz mitbestrahlt, während alle Kontrollproben vor Licht geschützt aufbewahrt wurden.

Die intensiv gelb gefärbten Mepacrinlösungen zeigten nach der Bestrahlung zunächst keine Veränderung, höchstens die cystein- und ascorbinsäurehaltigen, bei denen manchmal schon eine Farbvertiefung angedeutet war. Eine deutlich sichtbare Umfärbung der cystein- und ascorbinsäurehaltigen, bestrahlten Proben von Gelb über Orange bis Braun trat erst ein, wenn sie weiterhin dem Sonnenlicht exponiert wurden. Nach drei bis vier Wochen hatten sie eine schwarzbraune Farbe angenommen. Bestrahlte, reine Mepacrinlösungen und solche mit Glukosezusatz blieben unverändert gelb. Die unbestrahlten Kontrollproben mit Cystein und Ascorbinsäure änderten ihre Farbe wesentlich langsamer als die belichteten Lösungen. Auch ging die Umfärbung solcher Mepacrinlösungen mit Cystein- und Ascorbinsäurezusatz, wenn man sie nur bei Tageslicht stehenliess, nicht so schnell von statten, wie wenn vorher eine UV-Bestrahlung erfolgt war.

Die Umwandlung des Mepacrins stellt weder eine einfache Reduktion noch Oxydation dar, sondern es spielen sich dabei Oxydoreduktionsvorgänge ab, in deren Verlauf mehrere Stoffe entstehen. Mit Hilfe von Papierchromatogrammen liessen sich die braun gefärbten Umwandlungsprodukte vom unveränderten, gelb fluoreszierenden Mepacrin und anderen Substanzen abtrennen, die eine grüne oder blaue Fluoreszenz zeigten.

* Herrn Prof. Dr. Paul Wels, Greifswald, in Dankbarkeit und Verehrung zur Vollendung des 70. Lebensjahres gewidmet.

** Herrn Prof. Dr. Wirth und Herrn Stamer, Farbenfabriken Bayer, danken wir für die Überlassung von Atebrin (=Mepacrin) und Resochin (=Chloroquin) und Herrn Prof. Dr. Nieschulz, Promonta GmbH Hamburg, für das zur Verfügung gestellte Phenothiazin und Pacatal.

Literatur S. 593

Diese in den Modellversuchen beobachtete Reaktionsfähigkeit des Mepacrins steht mit seiner antiparasitären Wirksamkeit bei Malaria, Kokzidiose und Wurmkrankheiten gut in Einklang, weil dabei ebenfalls ein Eingriff in die Redoxvorgänge des Parasitenzellstoffwechsels im Sinne einer Schädigung desselben vor sich geht.

Das Malariaheilmittel Chloroquin verhielt sich *in vitro* ganz ähnlich wie Mepacrin. Die farblosen Chloroquinlösungen wurden auch braun gefärbt, wenn sie in Gegenwart von Cystein oder Ascorbinsäure UV-bestrahlt und danach noch längere Zeit dem Sonnenlicht ausgesetzt wurden. Deutlich ausgeprägt war die Verfärbung nach Ascorbinsäure-, etwas geringer nach Cystein- und noch weniger deutlich nach Glutathionzusatz. Abweichend vom Mepacrin färbte sich jedoch schon die Chloroquinlösung ohne Zusatz bei Belichtung rötlichbraun. Durch papierchromatographische Auftrennung des Gemisches liessen sich mehrere Bestrahlungsprodukte erkennen. Aus der Ähnlichkeit des Verhaltens von Mepacrin und Chloroquin lässt sich auf Analogien in der pharmakologischen Wirkung beider Substanzen schliessen.

An der Schweinehaut konnten in kurzfristigen Bestrahlungsversuchen keine einwandfreien Braunfärbungen durch Mepacrin oder Chloroquin erzeugt werden, weil offenbar der Zeitfaktor dafür ausschlaggebend ist. Bei Menschen, deren Haut im Verlauf einer Malariakur durch Mepacrin zunächst gelb gefärbt war, sind dagegen schwarzbraune Verfärbungen der Haut an denjenigen Körperpartien beobachtet worden, die längere Zeit intensiver Sonnenbestrahlung ausgesetzt worden waren[3]. Die in der Haut abgelagerten Malariamittel und ihre Umsetzungsprodukte gewähren hinreichenden Schutz vor Lichtschäden. Hierauf ist wohl vornehmlich ihre therapeutische Anwendung bei *Lupus erythematodes* begründet. Daneben wäre ausserdem an die Möglichkeit der Beeinflussung von gestörten Oxydoreduktionsprozessen in der erkrankten Haut zu denken.

Ganz anders als Mepacrin und Chloroquin reagierten bei Bestrahlung die in der Therapie viel benutzten Phenothiazine. Die als Anthelminticum gebräuchliche Grundsubstanz Phenothiazin war wegen ihrer schlechten Löslichkeit erst nach der Verarbeitung mit Acaciagummi zur Suspension für die Versuche geeignet. Bald nach Beginn der Bestrahlung mit UV-Licht trat ebenso wie nach längerem Einfluss des sichtbaren Lichtes in der Suspension des Phenothiazins ohne sonstigen Zusatz sowie in der glukosehaltigen eine Rosa- bis Rotfärbung infolge Photooxydation ein, die mit zunehmendem Säuregrad infolge besserer Löslichkeit des Phenothiazins immer deutlicher wurde. Zusätze von Cystein oder Ascorbinsäure bewirkten hierbei das Gegenteil, indem keine Oxydation des Phenothiazins durch Licht stattfand, sodass solche Suspensionen wie die vor Licht geschützten Proben farblos blieben. Als gefärbte Oxydationsprodukte des Phenothiazins sind Phenothiazon und Thionol zu nennen, deren Entstehung durch das starke Reduktionsvermögen der körpereigenen Substanzen Cystein und Ascorbinsäure verhindert werden kann.

Lösungen des Phenothiazinderivates Pacatal wurden bei Lichteinwirkung ebenfalls rot gefärbt, aber nicht bei Gegenwart von Ascorbinsäure oder Cystein.

Der Ausfall der Reagenzglasversuche lässt im Hinblick auf im Organismus mögliche Vorgänge die Annahme zu, dass die reduzierenden Substanzen der Haut im allgemeinen in der Lage sind, eine Photooxydation der Phenothiazine zu unterdrücken. Nur in Ausnahmefällen, etwa bei Anreicherung der Phenothiazine im Körper oder vielleicht bei Erlöschen der Schutzfunktion des Hautsulfhydrils infolge pathologischer Veränderungen wird man mit der Möglichkeit der Entstehung von Oxy-

lationsprodukten aus Phenothiazinen in der Haut rechnen müssen. Diese würden dann als eigentliche Ursache für Hauterkrankungen, die auf Photosensibilisierung oder Photoallergie zurückzuführen sind, in Betracht kommen.

Die Ergebnisse unserer Untersuchungen an drei Arzneimitteln spiegeln die verschiedenen Möglichkeiten der Umwandlung von Medikamenten durch Strahleneinwirkung wider. Indem sie einerseits zum pharmakologischen Wirkungsmechanismus dieser Stoffe, andererseits zu den durch ihre toxischen Nebenwirkungen verursachten Krankheitserscheinungen in Beziehung gebracht werden, bilden sie zugleich einen Beitrag zur Pharmakodynamik des Lichtes.

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[1] P. WELS, *Naunyn-Schmiedebergs Arch. exptl. Pathol. u. Pharmakol.*, 188 (1938) 265.
[2] G. URBAN, *Naunyn-Schmiedebergs Arch. exptl. Pathol. u. Pharmakol.*, 199 (1942) 345; 202 (1943) 337; 218 (1953) 86.
[3] PROF. KATSCH, Greifswald, Unveröffentlichte mündliche Mitteilung; G. URBAN, Eigene klinische Beobachtung.

Vergleich der Histaminbildung in Histidinlösungen durch Röntgenstrahlen und ultraviolettes Licht

Histidinlösungen, welche mit ultraviolettem (UV)-Licht, mit Kathodenstrahlen oder mit Röntgenstrahlen bestrahlt werden, senken den Blutdruck der narkotisierten Katze und erregen den isolierten Meerschweinchendarm wie Histamin[1-3] (Abb. 1.).

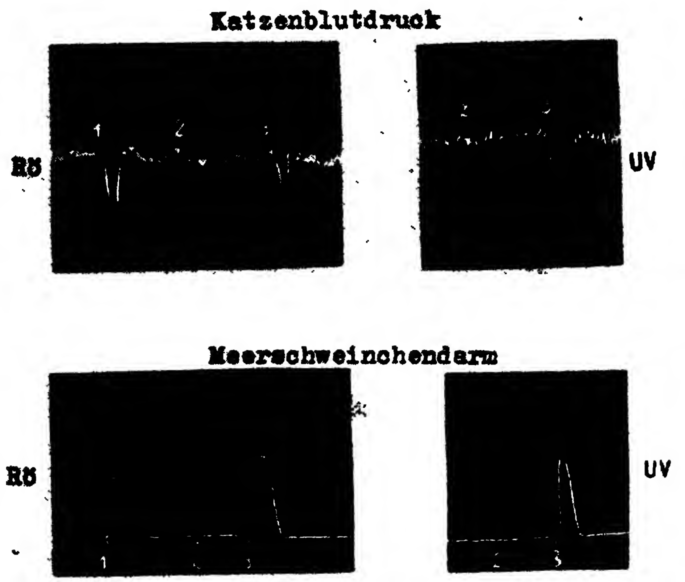


Abb. 1. Histaminwirkung bestrahlter Histidinlösungen am pharmakologischen Testobjekt. 1, Histamin; 2, Histidin, unbestrahlt; 3, Histidin, bestrahlt.

Literatur S. 600

Die durch das nichtionisierende UV-Licht gebildete histaminartig wirkende Substanz konnte schon vor längerer Zeit durch die Elementaranalyse[4], kürzlich auch durch die Papierelektrophorese[5], als Histamin identifiziert werden. Man durfte deshalb annehmen, dass der durch die ionisierenden Strahlen gebildete Wirkstoff ebenfalls mit Histamin identisch ist; bevor aber auch in diesem Falle Histamin nicht mit chemischen Methoden nachgewiesen worden ist, kann seine Bildung in Histidinlösungen durch ionisierende Strahlen nicht als sicher gelten. Ein solcher chemischer Histaminnachweis erscheint besonders deshalb erforderlich, weil sich die Wirkungsmechanismen des nichtionisierenden UV-Lichts und der ionisierenden Strahlen in wässrigen Lösungen erheblich voneinander unterscheiden.

Deshalb versuchte ich, in röntgenbestrahlten Histidinlösungen Histamin mit chemischen Methoden nachzuweisen. Zu diesem Zweck bestrahlte ich 1%-ige wässrige, neutral reagierende Histidinlösungen in luftdicht verschlossenen Glasschälchen mit hohen Röntgendosen (Röhrenstromstärke 20 mA; Röhrenspannung 160 kV; Bestrahlungsdauer 20 Std. Die mit einer Ionisationskammer ermittelte Strahlendosis betrug an der Oberfläche der Lösungen etwa 1,100 r/min, die eingestrahlte Gesamtdosis somit etwa 1,300 kr). Der am pharmakologischen Testobjekt bestimmte Histamingehalt dieser Lösungen war so gering, dass ich auch mit der sehr empfindlichen papierchromatographischen Methode erst dann verwertbare Ergebnisse erhielt, als ich den in den röntgenbestrahlten Histidinlösungen enthaltenen Wirkstoff stark anreicherte.

Abb. 2. Nachweis von Histamin in röntgenbestrahlten Histidinlösungen. 1, histaminartig wirkende Substanz aus Koessler-Hanke-Extrakten; 2, Histidin und Histamin zum Vergleich.

Diese Anreicherung erfolgte durch ein von Koessler und Hanke[6] angegebenes Verfahren für die Abtrennung von Histamin aus Histidinlösungen. Ich gewann damit aus grösseren Lösungsmengen sehr geringe Mengen eines histaminartig wirkenden Extraktes, der sich papierchromatographisch als eine Mischung von Histidin und Histamin erwies (Abb. 2).

Kürzlich hatte ich gefunden, dass die Histaminwirkung der röntgenbestrahlten Histidinlösungen grössenordnungsmässig verstärkt werden kann, wenn die Bestrahlung bei Gegenwart von Cystein erfolgt[7]. Aus solchen stark wirksamen Lösungen konnte ich durch das Koessler-Hanke-Verfahren nach Abdampfen des Lösungsmittels eine grössere Menge des histaminartig wirkenden Stoffes als trockenen Rückstand gewinnen; seine chemische Analyse konnte deshalb nun auch mit Methoden vorgenommen werden, die grössere Substanzmengen erfordern als die Papierchromatographie. Dabei zeigte sich, dass die Salze, welche sich aus den Lösungen dieses Trockenrückstandes durch Zusatz von Pikrinsäure oder Goldchlorid ausfällen liessen, die gleiche Ultrarotabsorption zeigten wie reines Histaminpikrat und reines Histaminchloraurat (Abb. 3 und 4). Die Elementaranalyse des aus dem Trockenrückstand

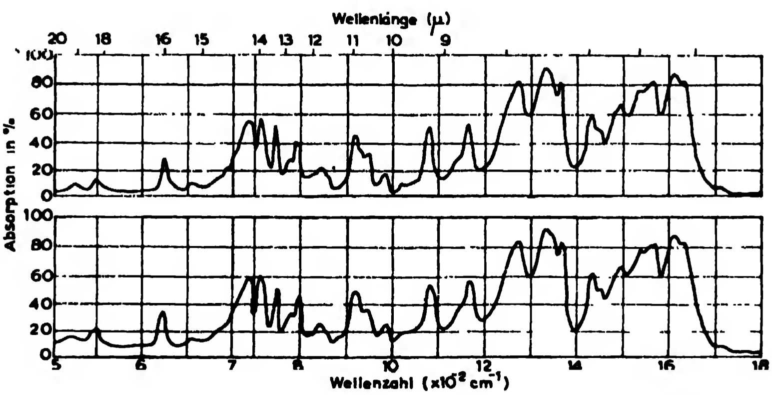


Abb. 3. Ultrarotspektren. Oben: Histaminpikrat; Unten: Pikrat der histaminartig wirkenden Substanz aus röntgenbestrahlten Histidinlösungen (Trockenrückstand aus Koessler-Hanke-Extrakten).

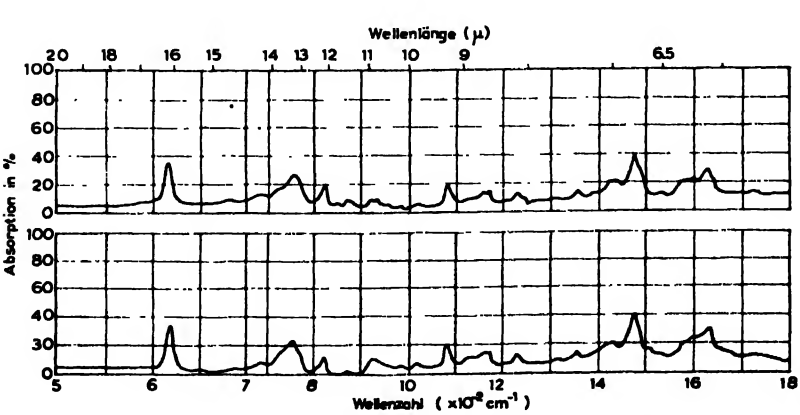


Abb. 4. Ultrarotspektren. Oben: Histaminchloraurat; Unten: Chloraurat der histaminartig wirkenden Substanz aus röntgenbestrahlten Histidinlösungen (Trockenrückstand aus Koessler-Hanke-Extrakten).

Literatur S. 600

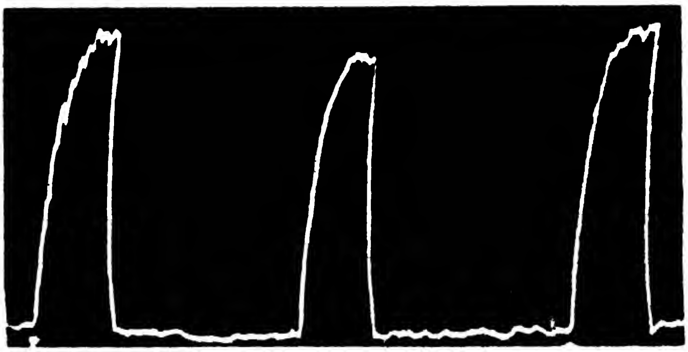
hergestellten Goldsalzes ergab eine völlige Übereinstimmung mit reinem Histaminchloraurat:

	C	H	N	Cl
Gefunden für das Chloraurat aus dem Trockenrückstand von Koessler-Hanke-Extrakten	7.40%	1.34%	5.18%	35.69%
Berechnet für Histaminchloraurat	7.59%	1.40%	5.31%	35.87%

Diese Versuchsergebnisse zeigen, dass in Histidinlösungen durch die ionisierenden Röntgenstrahlen der gleiche Wirkstoff gebildet wird wie durch das nichtionisierende UV-Licht, nämlich Histamin.

Die Tatsache, dass durch beide Strahlenarten das gleiche Reaktionsprodukt gebildet wird, besagt aber natürlich nicht, dass auch der Mechanismus der Strahlenwirkung und der damit in Zusammenhang stehende chemische Reaktionsmechanismus in beiden Fällen gleich ist. Was den Mechanismus der Strahlenwirkung betrifft, so muss man bei der UV- und Röntgenbestrahlung wässriger Lösungen sowohl mit direkten als auch mit indirekten Strahlenwirkungen rechnen. Es ergibt sich deshalb die Frage, in welchem Masse diese beiden Wirkungsmechanismen an der strahlenchemischen Histaminbildung beteiligt sind.

Zunächst zur direkten Strahlenwirkung. Für die Röntgenstrahlen liegen hier bereits die Befunde von Rajewski und Dose[8] vor. Diese Autoren haben nachgewiesen, dass durch Röntgenbestrahlung von trockenem, kristallisiertem Histidin, also bei Einhaltung von Versuchsbedingungen, unter denen indirekte Strahlenwirkungen ausgeschlossen werden können, Histamin gebildet wird. Ich konnte diesen Befund bestätigen und nun ein gleichartiges Ergebnis auch bei der Anwendung von UV-Licht erhalten. Histidin, welches als Trockensubstanz mit dem ungefilterten Licht einer Hg-



1 2 3

Abb. 5. In Substanz bestrahltes Histidin erregt den Meerschweinchendarm wie Histamin. Histidinbase, 4 h UV-bestrahlt oder 20 h röntgenbestrahlt. Herstellung 1%-iger Lösungen aus der bestrahlten Substanz. 1: 0.05 μg Histamin, 2: 0.1 ml Lösung von UV-bestrahltem Histidin, 3: 1.0 ml Lösung von röntgenbestrahltem Histidin.

Dampfquarzlampe bestrahlt worden war, wirkte nach seiner Auflösung in Wasser am isolierten Meerschweinchendarm in gleicher Weise histaminartig wie Lösungen, welche aus röntgenbestrahltem Histidin hergestellt worden waren (Abb. 5). — Der aus diesen Lösungen durch das Koessler-Hanke-Verfahren isolierte Wirkstoff liess

sich papierchromatographisch als Histamin identifizieren (Abb. 6). Diese Versuche zeigen, dass durch die direkten Wirkungen des UV-Lichtes und der Röntgenstrahlen aus Histidin Histamin gebildet werden kann. Man muss daraus schliessen, dass auch in Histidinlösungen eine direkte Histaminbildung grundsätzlich möglich ist.

Jetzt zu den indirekten Strahlenwirkungen. Sie sind nach den heute vorherrschenden Auffassungen u.a. daran zu erkennen, dass der Strahleneffekt in wässrigen Lösungen durch Sauerstoff und eine Reihe weiterer Stoffe beeinflusst wird. Ich prüfte deshalb, wie sich die strahlenchemische Histaminbildung in Sauerstoffatmosphäre und bei Gegenwart von Cystein verhält. Es zeigte sich ein deutlicher Einfluss beider Agentien auf die Strahlenwirkung (Abb. 7 und 8). An der Histaminbildung durch UV-Licht und durch Röntgenstrahlen in Histidinlösungen sind also indirekte Strahlenwirkungen beteiligt.

Abb. 6. Histaminbildung durch Bestrahlung von Histidin in Substanz. Histidinbase. UV: 4 h. Rö: 20 h. Koessler-Hanke (KH)-Extrakte, hergestellt aus 1%-igen Lösungen der bestrahlten Substanz. 1: Histidin, UV-bestrahlt. KH-Extrakt aus 25 ml Histidinlösung 2: Histidin, röntgenbestrahlt. KH-Extrakt aus 100 ml Histidinlösung 3: Histidin, unbestrahlt. KH-Extrakt aus 100 ml Histidinlösung + 10 μg Histamin, zum Vergleich. 4: 10 μg Histidin + 10 μg Histamin, zum Vergleich.

Aus den dargelegten Versuchsergebnissen geht eine bemerkenswerte Übereinstimmung in der Wirkung des UV-Lichtes und der Röntgenstrahlen insofern hervor, als sie zeigen, dass sowohl durch die direkten als auch durch die indirekten Wirkungen beider Strahlenarten aus Histidin Histamin gebildet werden kann. Man wird in wässrigen Histidinlösungen aber auch Unterschiede zwischen UV- und Röntgenwirkungen erwarten müssen, wenn man berücksichtigt, dass das UV-Licht von dem

Literatur S. 600

Histidin spezifisch, die Röntgenstrahlen dagegen unspezifisch absorbiert werden. Dieser Unterschied in der Strahlenabsorption hat nämlich zur Folge, dass das Verhältnis von direkten und indirekten Strahlenwirkungen zueinander bei UV- und Röntgenbestrahlung sehr verschieden sein muss. Es ist anzunehmen, dass direkte Strahlenwirkungen bei der photochemischen Histaminbildung eine grosse Rolle spielen, bei der radiationschemischen Histaminbildung dagegen kaum ins Gewicht fallen. In dem letzteren Falle kann die Histaminbildung praktisch nur durch indirekte

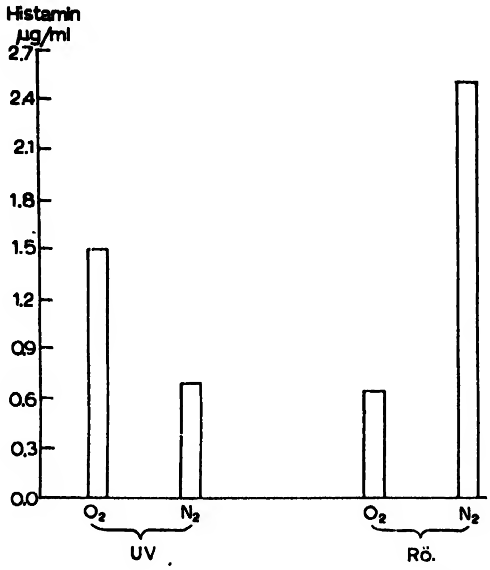


Abb. 7. Einfluss des Sauerstoffs auf die Histaminbildung durch UV-Licht und Röntgenstrahlen. 1 %-ige Histidinlösungen. UV: 30 min, Rö: 20 h.

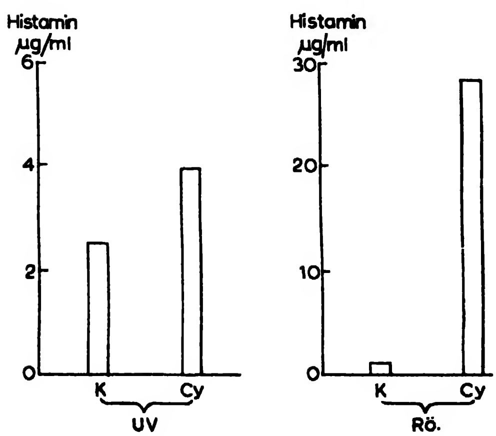


Abb. 8. Einfluss des Cysteins auf die Histaminbildung durch UV-Licht und Röntgenstrahlen. 1 %-ige Histidinlösungen. UV: 30 min; Rö: 20 h; K: Kontrolle; Cy: Cystein $3 \cdot 10^{-3}$ mol.

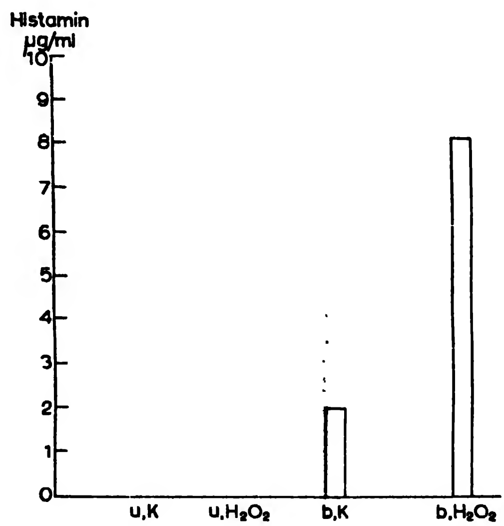


Abb. 9. Wasserstoffsuperoxyd verstärkt die Histaminbildung durch UV-Licht. 1 %-ige Histidinlösungen; UV: 30 min; u: unbestrahlt; b: bestrahlt; K: Kontrolle; H_2O_2: Wasserstoffsuperoxyd $6 \cdot 10^{-2}$ mol.

Strahlenwirkungen zustande kommen. In diesem Zusammenhang möchte ich auf die grossen Unterschiede zwischen der Wirkung des UV-Lichtes und der Röntgenstrahlen in den bereits dargelegten Versuchen aufmerksam machen, in denen ich den Einfluss des Sauerstoffs und des Cysteins auf die Histaminbildung prüfte. Der Sauerstoff wirkte bei der UV-Bestrahlung völlig anders als bei der Röntgenbestrahlung. Die Histaminbildung durch UV-Licht wurde durch Sauerstoff verstärkt, die Histaminbildung durch Röntgenstrahlen dagegen abgeschwächt (vgl. Abb. 7). Das Cystein wirkte bei der UV- und bei der Röntgenbestrahlung zwar grundsätzlich gleich, indem es sowohl die Histaminbildung durch UV-Licht als auch die Histaminbildung durch Röntgenstrahlen verstärkte. Der Grad dieser Verstärkungswirkung war bei beiden Strahlenarten jedoch sehr verschieden: die UV-Wirkung wurde durch Cystein nur wenig verstärkt, die Röntgenwirkung dagegen auf fast das dreissigfache erhöht (vgl. Abb. 8).

Es liegt natürlich nahe, sich auch über die bei der strahlenchemischen Histaminbildung stattfindenden chemischen Reaktionen gewisse Vorstellungen zu machen. Bei der direkten Strahlenwirkung liegen die Verhältnisse insofern einfach, weil sie als eine unmittelbare Decarboxylierung des Histidins verstanden werden kann. Verwickelter sind die Verhältnisse bei der indirekten Histaminbildung. Hier kommen vermutlich sehr verschiedene Reaktionsmechanismen in Frage. Einer dieser Mechanismen ist offenbar oxydativer Natur. Soweit ich sehe, sind OH-Radikale dabei massgebend beteiligt. Dies geht daraus hervor, dass OH-Radikale, welche durch die photochemische Zersetzung von H_2O_2 erzeugt werden, ebenfalls eine starke Histaminbildung bewirken (Abb. 9). -- Dies Versuchsergebnis kann einerseits die Histaminbildung in röntgenbestrahlten Histidinlösungen erklären, denn dabei werden ja durch Wasserspaltung OH-Radikale gebildet; es kann andererseits vielleicht aber auch die Verstärkungswirkung des Sauerstoffs auf die Histaminbildung durch UV-Licht verständlich machen (vgl. Abb. 7). Bekanntlich wird ja durch UV-Bestrahlung H_2O_2 nicht nur gespalten und somit zerstört, sondern bei Gegenwart von Sauerstoff in wässrigen Lösungen auch neu gebildet. Das photochemisch neugebildete H_2O_2 muss aber der Spaltung durch das UV-Licht ebenso unterliegen wie das in den zuletzt dargelegten Versuchen vor der UV-Bestrahlung zu den Histidinlösungen hinzugesetzte H_2O_2. Folglich müssen in den UV-bestrahlten Lösungen bei Sauerstoffgegenwart auch OH-Radikale entstehen; sie könnten für den Verstärkungseffekt des Sauerstoffs auf die photochemische Histaminbildung ursächlich verantwortlich sein. Wenn man die in der Literatur formulierten Reaktionsmechanismen für die Einwirkung von OH-Radikalen auf organische Verbindungen berücksichtigt[9-11], dann halte ich es für möglich, die Histaminbildung durch OH-Radikale formelmässig in der nachstehend beschriebenen Weise darzustellen.

$$R-\overset{\displaystyle H}{\underset{\displaystyle NH_2}{C}}-COOH \;+\; OH\cdot \longrightarrow R-\overset{\displaystyle H}{\underset{\displaystyle NH_2}{C}}-COO\cdot \quad H_2O$$

$$R-\overset{\displaystyle H}{\underset{\displaystyle NH_2}{C}}-COO\cdot \longrightarrow R-\overset{\displaystyle H}{\underset{\displaystyle NH_2}{C}}\cdot \;+\; CO_2$$

Literatur S. 600

$$\mathrm{R{-}\overset{\displaystyle H}{\underset{\displaystyle NH_2}{\overset{|}{\underset{|}{C}}}}\cdot} \quad + \quad \mathrm{H_2O} \longrightarrow \mathrm{R{-}\overset{\displaystyle H}{\underset{\displaystyle NH_2}{\overset{|}{\underset{|}{C}}}}{-}H} \quad + \quad \mathrm{OH\cdot}$$

Ein zweiter Mechanismus der indirekten Histaminbildung, der bei Gegenwart von Cystein zur Auswirkung kommt, beinhaltet neben oxydierenden auch reduzierende Reaktionen, wie aus der verstärkenden Wirkung des Cysteins hervorgeht. Für die Erklärung dieser Cysteinwirkung in UV-bestrahlten Histidinlösungen habe ich kürzlich folgenden Reaktionsmechanismus vorgeschlagen[12]:

$$\underset{\textit{Aminosäure}}{\mathrm{R\cdot \underset{\displaystyle NH_2}{\underset{|}{CH}}\cdot COOH}} \xrightarrow[-\mathrm{H_2}]{\substack{\text{Peroxydartige Zwischenstufe der}\\ \text{Thioloxydation}\ \downarrow}} \mathrm{R\cdot \underset{\displaystyle NH}{\underset{\|}{C}}\cdot COOH} \xrightarrow[\mathrm{CO_2}]{\text{spontan}\ \downarrow} \mathrm{R\cdot \underset{\displaystyle NH}{\underset{\|}{CH}}} \xrightarrow[+\mathrm{H_2}]{\text{Thiol}\ \downarrow} \underset{\textit{Amin}}{\mathrm{R\cdot \underset{\displaystyle NH}{\underset{|}{CH_2}}}}$$

Wahrscheinlich lässt er sich auch auf die Cysteinwirkung in röntgenbestrahlten Histidinlösungen anwenden.

Die hier nur kurz erwähnten Vorstellungen über den chemischen Reaktionsmechanismus bei der strahlenchemischen Histaminbildung in Histidinlösungen bedürfen noch einer Stützung durch weitere Versuche und müssen vielleicht auch in der einen oder anderen Weise noch abgeändert werden. Ich hoffe, dass gerade die vergleichende Anwendung des UV-Lichtes und der Röntgenstrahlen es ermöglicht, die Grundvorgänge bei der strahlenchemischen Histaminbildung besser kennen zu lernen.

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[1] F. ELLINGER, *Naunyn-Schmiedeberg's Arch. exptl. Pathol. Pharmakol.*, 136 (1928) 129; 153 (1930) 120; 161 (1931) 703.

[2] P. HOLTZ, *Pflügers Arch. ges. Physiol.*, 226 (1931) 558.

[3] J. P. BECKER UND S. FREYTAG, *Pflügers Arch. ges. Physiol.*, 231 (1933) 26.

[4] P. HOLTZ, *Klin. Wochschr.*, 41 (1933) 1513, *Naunyn-Schmiedeberg's Arch. exptl. Pathol. Pharmakol.*, 175 (1934) 97.

[5] G. PFENNIGSDORF UND H. SCHREIBER, *Probleme und Ergebnisse aus Biophysik und Strahlenbiologie* II, Akademie Verlag, Berlin, 1960, p. 170.

[6] K. H. KOESSLER UND M. T. HANKE, *J. Biol. Chem.*, 39 (1919) 521, 50 (1922) 131.

[7] K. FLEMMING, *Naunyn-Schmiedeberg's Arch. exptl. Pathol. Pharmakol.*, 238 (1960) 235.

[8] B. RAJEWSKY UND K. DOSE, *Z. Naturforsch.*, 12 b (1957) 384.

[9] W. A. WATERS, *Chemistry of Free Radicals*, Oxford University Press, 2nd Ed., 1948.

[10] C. WALLING, *Free Radicals in Solution*, John Wiley and Sons, New York, 1957.

[11] R. MAXWELL, D. C. PETERSON UND N. E. SHARPLESS, *Radiation Research*, 1 (1954) 530.

[12] K. FLEMMING, *Naunyn-Schmiedeberg's Arch. exptl. Pathol. Pharmakol.*, 236 (1959) 27.

Photochemische Veränderungen in wässrigen Lösungen von Histidin

Ausgehend von den grundlegenden Arbeiten von Ellinger[1] und Holtz[2] haben wir die Veränderungen in wässrigen Lösungen von Histidin bei UV-Bestrahlung weiter untersucht.

Versuchsanordnung: *l*-Histidinmonohydrochlorid wurde in 5%-iger wässriger Lösung mit dem Gesamtspektrum eines Quarz-Quecksilber-Hochdruckbrenners des VEB Berliner Glühlampenwerk vom Typ S 250 unter Verwendung eines 2 cm starken Wasserfilters in einer Quarzküvette bestrahlt. Die Schichtdicke betrug 0.5 cm, der Brennerabstand 20 cm. Der Brenner wurde mit Gleichspannung betrieben, die Intensität lag im Mittel bei $3.27 \cdot 10^4$ erg·cm^{-2}·sec^{-1}. Die Lösungen wurden während der Bestrahlung ständig entweder mit Sauerstoff oder mit Stickstoff durchströmt. Das Bestrahlungsprodukt wurde mittels Hochspannungspapierelektrophorese (Pyridin–Essigsäure-Puffer; pH 4.0) und Papierchromatographie (Lösungsmittel: *tert.* Butanol–Ameisensäure–Wasser im Verhältnis 695 : 10 : 295 und Phenol–Wasser im Verhältnis 775 : 225 - 1% konz. NH_3) aufgearbeitet. Es wurden nur ninhydrinpositive Verbindungen nachgewiesen, so dass wir über das mögliche Auftreten anderer Substanzen (wie z.B. von Imidazolacetaldehyd[3]) die diese Reaktion nicht geben, keine Aussage machen können.

Veränderungen in O_2-durchströmten Lösungen: Wie wir schon früher mitgeteilt haben, ist nach einer Bestrahlungszeit von 4 bis 6 Stunden etwa 1/1000 der eingesetzten Histidinmenge zu Histamin dekarboxyliert[4]. Bei weiterer Bestrahlung steigt der Histamingehalt in der bestrahlten Lösung zwar weiter an, es wird aber die Zunahme an Histamin je Zeiteinheit kleiner. Es strebt also mit anderen Worten der Histamingehalt in der bestrahlten Lösung einem Sättigungswert zu. Hieraus war zu schliessen, dass auch das entstandene Histamin weiter verändert wird. Bei UV-Bestrahlung von Histamindihydrochlorid in wässriger Lösung erhielten wir entsprechend eine Substanz, die als β-Alanin identifiziert wurde. Die gleiche Verbindung ist auch in Lösungen von bestrahltem Histidin nachzuweisen. Während Histamin also primär, wenn auch sicher über eine Reihe von Zwischenstufen[5] aus Histidin entsteht, stellt das β-Alanin in bestrahlter Histidinlösung wahrscheinlich ein Folgeprodukt dieser primären Reaktion dar. Wesentlich früher als Histamin tritt aber eine andere ninhydrinpositive Verbindung auf, die wir als Asparaginsäure bestimmen konnten. Diese Substanz wurde von Elpiner[6] auch nach der Einwirkung von Ultraschall auf Histidinlösung erhalten. Sie muss wie das β-Alanin durch oxydative Aufspaltung des Imidazolringes entstanden sein. Eine solche Ringsprengung ist für Kathodenstrahlen bewiesen worden[7]. Wir fanden nach einer Bestrahlungszeit von 60 Stunden ($58.6 \cdot 10^8$ erg·cm^{-2}) 158 μg Asparaginsäure/ml bestrahlter Histidinlösung. Die gebildete Aminosäure strebt ähnlich wie Histamin einem Sättigungswert zu. Um die Frage zu klären, in welcher Weise Asparaginsäure neben dem bekannten Abbau zu Acetaldehyd weiter verändert wird, wurde die Reinsubstanz in wässriger Lösung bestrahlt. Dabei wurde die um ein C-Atom ärmere Verbindung, und zwar das α-Alanin, das demnach durch Dekarboxylierung der β-ständigen Karboxylgruppe entstanden sein muss, nur in Spuren erhalten. Wir haben aber in Lösungen von bestrahltem Histidin eine unver-

Literatur S. 604

hältnismässig stärkere Bildung von α-Alanin und auch von Glycin beobachtet. Es liegt deshalb die Annahme nahe, dass diese Aminosäuren direkt aus dem Histidinmolekül durch Absprengung der Seitenkette, bzw. eines Teiles der Seitenkette entstehen. Die entsprechenden Amine, das Äthyl- bzw. Methylamin, deren Bildung Pfordte[8] bei UV-Bestrahlung der Aminosäuren sichern konnte, fanden wir mit unserer Methodik bisher nur nach Röntgenbestrahlung. In äusserst geringer Menge entsteht neben Asparaginsäure das nächst höhere Homologe, die Glutaminsäure, die ja ein bekanntes Zwischenprodukt beim Abbau des Histidins *in vivo* ist. Auch hier muss eine Ringsprengung angenommen werden. Es ergibt sich demnach für die in O_2 gesättigter wässriger Histidinlösung bei UV-Bestrahlung primär bzw. sekundär entstehenden ninhydrinpositiven Verbindungen das folgende Schema:

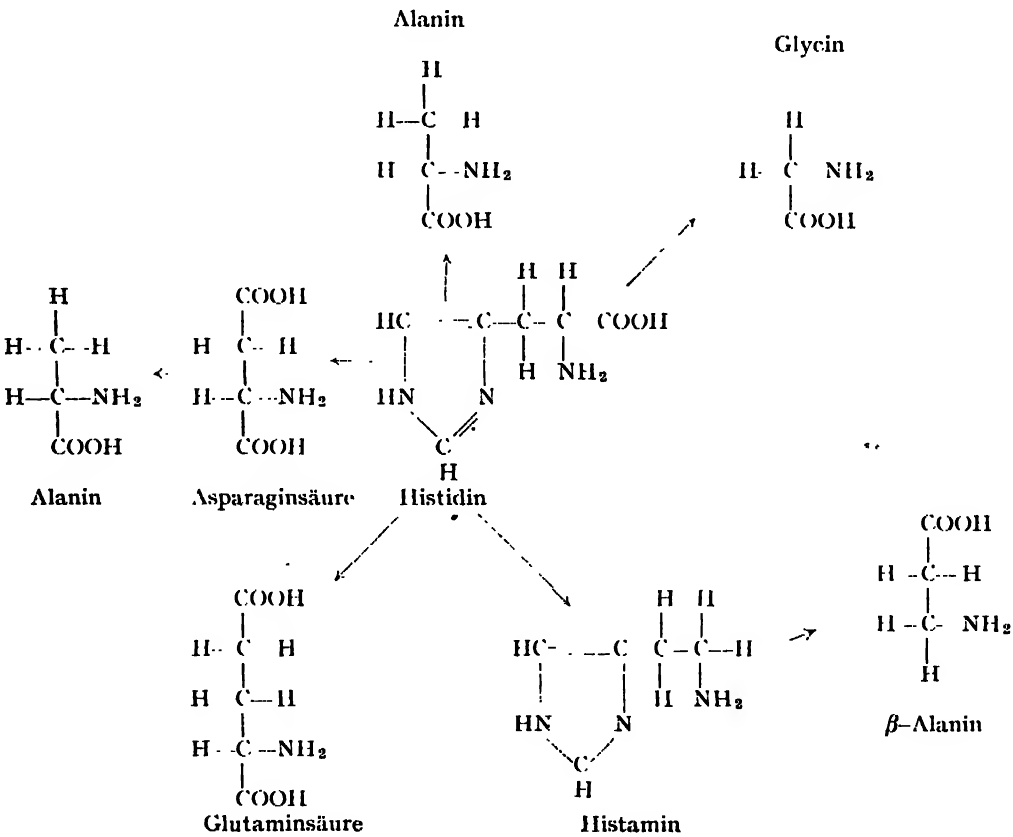

Abb. 1.

Wird die gleiche Probe in N_2-gesättigter Lösung bestrahlt, so kommt es nur nach sehr langen Bestrahlungszeiten zu einer geringen Bildung von Asparaginsäure. Ebenso ist die Histaminbildung sehr viel geringer. In gleicher Weise entsteht β-Alanin in N_2-gesättigter Histaminlösung nur in sehr geringem Ausmass. Dagegen wird die Bildung von Alanin und Glycin nach unseren Untersuchungen nicht erkennbar beeinflusst.

Neben dieser Bildung ninhydrinpositiver Verbindungen kommt es bei UV-Bestrahlung sowohl von Histidin- als auch von Histaminlösung, wie auch von anderen Auto-

ren beschrieben, zu einer gelben bis gelbbraunen Verfärbung, die wir durch laufende Messungen der Extinktion am Pulfrich-Photometer in Abhängigkeit von der eingestrahlten Energie bestimmt haben. Die Verhältnisse für bestrahlte Histidinlösung sind in der Abb. 2 dargestellt. Es ist zu ersehen, dass die Geschwindigkeit der Farbstoffbildung bei der N_2-gesättigten Lösung ständig ansteigt. Vielleicht kann man

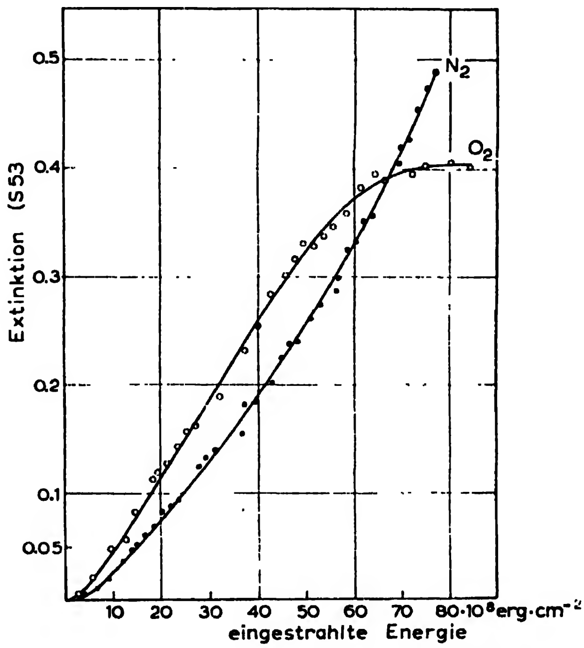

Abb Farbstoffbildung bei U.V.-Bestrahlung von wässriger Histidinlösung.

hieraus schliessen, dass erst eine andere Reaktion ablaufen muss, die dann das Entstehen des Farbstoffes ermöglicht. Die O_2-durchströmte Lösung zeigt anfangs ein ähnliches Verhalten, nach längerer Bestrahlung wird jedoch die Geschwindigkeit geringer, und die Farbstoffbildung strebt einem Sättigungswert zu. Bei der in O_2-Atmosphäre bestrahlten Lösung kommt es anscheinend im weiteren Verlauf der Bestrahlung wieder zu einem Abbau des Farbstoffes im Sinne einer Ausbleichung. Der Farbstoff ist nicht einheitlich. Es lassen sich elektrophoretisch zwei Banden, die im elektrischen Feld zur Kathode wandern, unterscheiden. Das Verhalten der Extinktion bestrahlter wässriger Lösungen von Histamin entspricht prinzipiell dem in der Abb. 2 dargestellten Verlauf. Elektrophoretisch lassen sich hier drei verschiedene Fraktionen feststellen. Nach längerer Bestrahlung kommt es zur Bildung eines feindispersen, schwarzen Niederschlages, der sich in Alkali löst.

Es ist uns bisher leider nicht möglich eine Aussage über die chemische Natur der Farbstoffe bzw. des Niederschlages zu machen.

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Literatur S. 604

[1] F. ELLINGER, *Arch. exptl. Pathol. Pharmakol.*, 136 (1928) 129; 153 (1930) 120.
[2] P. HOLTZ, *Arch. exptl. Pathol. Pharmakol.*, 175 (1934) 97.
[3] P. SZENDRÖ, *Pflügers Arch. ges. Physiol.*, 228 (1931) 743.
[4] G. PFENNIGSDORF UND H. SCHREIBER, *Probleme und Ergebnisse aus Biophysik und Strahlenbiologie II*, Akademie Verlag, Berlin, 1960.
[5] P. HOLTZ UND R. HEISE, *Naturwissenschaften*, 25 (1937) 14.
[6] I. JE. ELPINER, I. B. SBARSKIU UND W. N. CHARLAMOWA, *Ber. Akad. Wiss. U.D.S.S.R.*, 73 (1950) 1255.
[7] D. S. BHATIA UND B. E. PROCTOR, *Biochem. J.*, 49 (1951) 550.
[8] K. PFORDTE UND G. LEUSCHNER, *Strahlentherapie*, 113 (1960) 140.

Changes produced by ultraviolet light in DNA

Hollaender, Greenstein, and Jenrette found in 1941 that the viscosity of dilute solutions of DNA was decreased by UV irradiation[1]. This change could be the result of either main chain scission or of increased coiling of the DNA molecule and can be distinguished only by making other measurements in addition to viscosity. It is also possible for UV irradiation to crosslink DNA molecules in the solid state, as reported by Shugar and Baranowska[2] and by Setlow and Doyle[3]. It is interesting to note that both double chain scission and crosslinking are observed when DNA is irradiated with β- and γ-rays in fibre form[4].

We have irradiated dilute solutions of DNA (0.01–0.02%) in 0.2 M sodium chloride plus 0.015 M sodium citrate with either of two low pressure 44-W mercury resonance lamps, the first in a vycor envelope and the second in a quartz envelope. The light intensity was $2 \cdot 10^{-8}$ $Nh\nu/cm^3/sec$ or 10^5 ergs/sec of 254 mμ. The DNA sample used was essentially protein free and prepared from salmon sperm by the Kay-Simmons-Dounce technique. This sample had the following characteristics:

Molecular weight	$9.6 \cdot 10^6 \pm 1 \cdot 10^6$	(by light scattering)
Radius of gyration	$= 2840$ Å ± 200	
Sedimentation constant	$= 24.7 \cdot 10^{-13}$	
Intrinsic viscosity	$= 110 \pm 5$ dl/g	

As first shown by Doty[5], DNA in dilute solution has the properties of a stiffened random coil, and relationships between the viscosity, molecular weight, and radius of gyration of the macromolecule have been found which intact DNA from many sources obey. Our sample obeyed the set of relationships:

$$Mls = 1.14\ R_z^2 \qquad \text{where } R_z \text{ is radius of gyration in Ångstroms.}$$
$$[\eta] = 1.26 \cdot 10^{-5}\ R_z^2 \qquad \text{where } [\eta] \text{ is intrinsic viscosity in dl/g}$$

During irradiation either nitrogen or oxygen was bubbled through the DNA solution after which the light scattering measurements and viscosity measurements in a couette viscometer were performed.

In the absence of oxygen the first effect of 254-mμ light is to lower the viscosity with no change in the molecular weight but with a change in the radius of gyration. We call this effect "coiling". The initial effect of UV light of the doses we must use to observe a change is visualized to be one of breaking of H bonds. This causes a disorganization of the secondary structure of DNA and leads to increased coiling.

Another measure of this is the change of hyperchromic effect due to UV irradiation. If one heats DNA at 95° for 10 min in saline citrate solution, the absorbance increases by about 15% due to the thermal disruption of the double helical coil configuration. In UV-treated DNA, the relative increase in absorption is found to decrease with increasing UV dose. That is, UV has diminished the heat effect. The destruction of a few pyrimidine bases is not sufficient to account for the changes in radius of gyration and hyperchromic effect observed.

If oxygen is present with 254-mμ irradiation the molecular weight as well as the radius of gyration fall with dose. The viscosity falls at a faster rate than without oxygen present. The rate of coiling is also increased, and the coiling is accompanied by main chain scission, which we shall call "chopping".

Using the quartz lamp, under nitrogen we observe immediate double chain scission as well as coiling. Correlating the viscosity, molecular weight, and radius of gyration, we find that the expression for a stiff random coil is not obeyed. However, when oxygen is introduced, at early stages of irradiation the chopping reaction predominates, and these relatonships are obeyed.

Since the number of breaks is greatly enhanced by oxygen with unfiltered light, *i.e.* an oxygen effect, we investigated the role of several protective agents in the DNA solution during UV irradiation. Substances such as 10^{-1} *M* glucose, 10^{-3} *M* glutathione, and 10^{-4} *M* dimethyl aniline did protect against the oxygen effect by reducing the number of breaks per molecule. As Dr. Gray pointed out[6], the biological oxygen response of UV is negative while the chemical response is positive. It may be that cell constituents act as protective agents which protect against the oxygen effect.

When DNA is treated with propylene oxide or other monofunctional alkylating agents, the principle effect observed is one of coiling of the DNA by a mechanism not yet understood. When DNA is treated with OH radicals from H_2O_2, the principle effect produced is chopping.

The effect of UV radiation of relatively high doses on DNA thus can be:

(*1*) to break hydrogen bounds and render the chain more flexible (referred to as *coiling*).

(*2*) to break the double chain and reduce molecular weight in proportion with viscosity (referred to as *chopping*).

(*3*) to form an insoluble gel (referred to as *crosslinking*).

I would like to express my thanks to the National Research Council, Division of Biology and Agriculture, for a travel grant to attend this Congress.

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[1] A. HOLLAENDER, J. P. GREENSTEIN AND W. V. JENRETTE, *J. Natl. Cancer Inst.*, 2 (1941) 22.
[2] D. SHUGAR AND J. BARANOWSKA, *Nature*, 165 (1960) 33.
[3] R. SETLOW AND B. DOYLE, *Biochim. Biophys. Acta*, 15 (1954) 117; R. SETLOW, *Biochim. Biophys. Acta*, 39 (1960) 180.
[4] P. ALEXANDER, J. T. LETT, H. MOROSON AND K. A. STACEY, *Suppl. to Intern. J. Radiation Biol.*, (1960).
[5] P. DOTY, *J. Cellular Comp. Physiol.*, 49 (Suppl. 1) (1957) 27.
[6] L. H. GRAY, this Congress.

Photochemistry of 4-aminopyrimidines

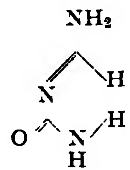
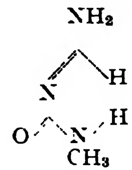
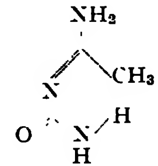
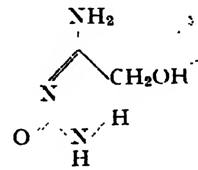
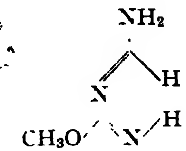
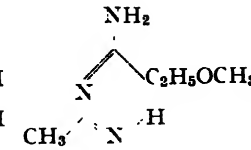
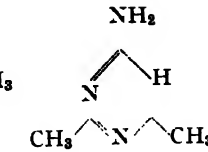
The following is a brief summary of current attempts to elucidate the nature of the photoproducts of 5-substituted cytosine nucleotides in irradiated nucleic acids, partly through comparative studies on other more readily available 4-aminopyrimidines.

Of a variety of 4-aminopyrimidines, cytosine and its N1-substituted derivatives, particularly nucleosides and nucleotides, are somewhat unusual in their behaviour in aqueous media under the influence of ultraviolet irradiation (2537 Å) in that a water molecule is taken up at the 5,6 double bond with resultant formation of 5-hydro-6-hydroxy derivatives. These photoproducts are relatively unstable and spontaneously revert to the parent compound in the dark by elimination of the water molecule[1-3]. The quantum yields for a number of these derivatives at various pH values have been reported[3]. More precise measurements taking into account the rates of the reverse reactions have now shown that for a given derivative the lower the quantum yield, the less stable the photoproduct, *i.e.* the more rapid is the rate of the reverse reaction. During irradiation an equilibrium state is established between the forward and reverse reactions and this is gradually shifted as a result of formation of secondary photoproducts via side reactions. For cytosine, (d)pC and (d)pCp, the rates of the reverse reactions are so high that it is difficult to observe them. The proposal of Wang[4] that irradiation of the foregoing compounds is due to "phototautomerization" is erroneous, due to his overlooking the fact that (*a*) some dihydropyrimidines, *viz.* those of cytosine, may exhibit selective absorption[5] to the red of 2300 Å; (*b*) in 1-methylcytosine and cytosine nucleosides the number 1 nitrogen is substituted by a methyl group or a sugar.

For the other 4-aminopyrimidines hitherto examined the photochemical reaction is a different one, involving a rearrangement of the pyrimidine rings and usually most readily detected by the appearance of a new absorption band in the ultraviolet to the red of that exhibited by the parent substance. In an anhydrous medium (ethanol) a similar reaction is exhibited by cytosine and 1-methylcytosine, but not by cytosine nucleosides and nucleotides. As can be seen from the accompanying Table, this type of reaction is exhibited by some derivatives only in an aqueous medium and by others only in an anhydrous medium. There is some evidence that the solvent itself does not participate in this reaction: *e.g.* 2,6-dimethyl-4-amino pyrimidine undergoes the same reaction in water, ethanol or hexane. The photoproduct of 5-methylcytosine in alcohol, upon transfer to aqueous medium, is spontaneously transformed to the photoproduct formed by irradiation in aqueous medium. In aqueous medium at pH 9, cytosine also exhibits this type of reaction in contrast to its behaviour at neutral pH; it is consequently of interest that this is the optimum pH for the photochemical transformation of 5-methylcytosine and 5-hydroxymethylcytosine.

Finally it should be noted that nucleotides of 5-substituted cytosines not only differ in behaviour from the corresponding bases but, in place of photochemical addition of water to the 5,6 double bond, also undergo some type of rearrangement without pyrimidine ring rupture[6], or opening of the pyrimidine ring.

TABLE I

Compound							
Principal long-wave absorption maximum in water at pH 7 (Å)	2670	2740	2735	2700	2700	2720	2660
Principal absorption maximum of photo-product in water (Å)	2400	2400	2850	2820	3020	3050	2950
Reversibility (Å)	50%	50%	nil	nil	partial	100%	nil
Principal absorption maximum of photo-product in ethanol (Å)	3000	3100	3100	—	Only slow drop in absorption	Only slow drop in absorption	2950
Reversibility	partial	partial	nil	—	—	—	nil
Remarks	At pH 9 in water there is slight increase in absorption to red of 2700 Å, but no formation of maximum.	—	Reaction optimal at pH 9 in H_2O. Photoproduct formed in alcohol, on transfer to aqueous medium, spontaneously transforms to photoproduct formed in water.	Reaction optimal at pH 9 in water.	—	Reversibility gives parent compound with identical physico-chemical and biological properties. In acid medium reaction is irreversible.	Photoproducts identical in water, ethanol or hexane. Heating of photoproduct in water results in rearrangement to a similar compound.

It is clear that spectral techniques alone are inadequate for elucidating the nature of these photoproducts which are of so much importance in the interpretation of photobiological effects[7,8], the more so in that the photochemical reaction for 2-methoxy-cytosine is partially reversible, while for the pyrimidine component of vitamin B_1 it is completely reversible physico-chemically and biologically.

A new type of irradiation source has therefore been developed to prepare sufficiently large quantities of photoproducts for chemical and physico-chemical analyses. The best results to date have been with 2,6-dimethyl-4-aminopyrimidine; in 0.02 *M* phosphate buffer at pH 8–9 the photoproduct of this compound crystallized spontaneously. Its m.p. is 201–202° as compared to 180–181° for the parent substance and its chemical composition is identical. Heating of this photoproduct in water results in its transformation to a water-soluble substance with a m.p. of 134–135° and a chemical composition suggesting deamination; however titrimetric, but not spectral, procedures show that some type of amino group is still present. Although kinetic data for some compounds suggest dimerization, attempts to estimate the molecular weights of these two products by a variety of methods have thus far been unsuccesful because of association with the solvents used. The infra-red spectra of both compounds exhibit a narrow, very intense characteristic band at 2200 cm^{-1} which can only be due to the following:

$$R-\overset{+}{N}\equiv\overset{-}{C} \text{ or } R-N=\overset{+}{N}=\ddot{N} \text{ or its equivalent } R-C-\overset{+}{N}-\ddot{N}$$

The above is only a very sketchy outline of the data accumulated on the photochemistry of 4-aminopyrimidines, which we believe will lead to the elucidation of the photochemical reaction mechanisms of 5-substituted cytosine derivatives in nucleic acids, a problem of considerable significance in the photochemistry and genetics of the T-even bacteriophages[6,8]. The findings should also be of some interest to the synthetic organic chemist.

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1 R. L. SINSHEIMER, *Radiation Research*, 6 (195[illegible]) 121.
2 D. SHUGAR AND K. WIERZCHOWSKI, *Biochim. Biophys. Acta*, [illegible] (195[illegible]) 657
3 K. L. WIERZCHOWSKI AND D. SHUGAR, *Biochim. Biophys. Acta*, 2[illegible] (1957) 355.
4 S. Y. WANG, *Nature*, 184 (suppl. 4) (1959) 184.
5 C. JANION AND D. SHUGAR, *Acta Biochim. Polon.*, 7 (1960) 309
6 K. L. WIERZCHOWSKI AND D. SHUGAR, *Acta Biochim. Polon.*, 7 (1960) 63
7 D. SHUGAR AND K. L. WIERZCHOWSKI, *Postepy Biochemii*, 4 (suppl.) (1958) 243.
8 D. SHUGAR, in E. CHARGAFF AND J. N. DAVIDSON (eds.), *The Nucleic Acids*, Vol. III, Chapter 30, Academic Press, New York, 1960.

Photochemistry of pyrimidine residues in oligonucleotides and in single- and double-stranded polynucleotides

The development during the past few years of chemical and enzymatic techniques for the synthesis of homo- and hetero-polymers of nucleotides has made available model single- and twin-stranded polynucleotides of various chain lengths, base composition and base sequences. Such models would be expected to prove extremely useful for analyses of the photochemical behaviour of various constituents in nucleic acid chains, and initial studies have amply confirmed this assumption[1,2]. Some simplification is also introduced by the fact that the radiation resistance of the purine residues in polymers is so much greater than that of pyrimidines unsubstituted in the 5-position that spectral modifications resulting from irradiation are due almost exclusively to the latter.

The dominant reaction in irradiated poly-U and poly-C is the uptake of a water molecule at the 5,6 double bond of the uracil and cytosine rings with resultant formation of 5-hydro-6-hydroxy derivatives. These are capable of reverting, in the dark, to the parent compound, usually by heating. In addition to this reaction, there is another which involves interaction between adjacent pairs of pyrimidine bases and which is irreversible by heating. The net result is that thermal reactivation (TR) for poly-U varies from 80% for a dinucleotide to 65% for highly polymerized chains; for poly-C it is from 90–85%. The irreversible reaction could conceivably be due in part to the formation of 5-hydroxy-6-hydro photoproducts, which would not be expected to exhibit heat reversibility[3,4]; but this has been excluded by acid hydrolysis of irradiated poly-U and demonstration of the absence of such photoproducts. The second, irreversible, reaction is exhibited also by thymine residues in poly-T with a quantum yield for some of the residues considerably in excess of that for Tp and again suggestive of formation of cross-links between adjacent residues[2].

No oxygen effect, such as has been observed for uracil, orotic acid and thymine[5], was found for poly-T or any of the other oligonucleotides investigated.

In dinucleotides where a uracil or cytosine residue is flanked by a purine ring (*e.g.* ApUp, ApCp, GpCp), the former undergoes exclusively hydration at the 5,6 double bond and subsequent reversibility is from 90–100%. In trinucleotides containing two purine residues and one uracil, the %TR for the latter is somewhat reduced, for reasons as yet unclear.

The accompanying Table I presents the quantum yields, Φ, in moles/einstein; the 1st order rate constants, k, for the reverse reaction at neutral pH; the activation energy, E, for the reverse reaction; and the maximum %TR for uracil and cytosine residues in various oligo- and polynucleotides.

In single-stranded chains, particularly small oligonucleotides, spectral methods may be readily used for following the reaction[1] by assuming, to a first approximation, that hyperchromicity decreases linearly with the course of the reaction. For twin-stranded chains this is no longer possible. For example, in the complex poly-A–poly-U the decrease in absorption due to photolysis of the uracil residues is almost completely masked by the resultant decrease in interchain hyperchromicity; photolysed poly-U

References p. 611

TABLE 1

REACTION CONSTANTS FOR PHOTOCHEMISTRY OF URACIL AND CYTOSINE RESIDUES IN POLYNUCLEOTIDES

	Up residue in						*Cp residue in*					
	UpUp	*poly-U*	*ApUp*	*CpU*	*GpUpA* *GpApU* *UpGpA*	*poly-A–poly-U complex*	*CpCp*	*poly-C*	*ApCp* *ApApCp*	*CpU*	*GpCp* *GpCpGpCp*	*poly-I–poly-C complex*
$\Phi \times 10^3$	70[a] 8.0	55[b] 8	7.0	10	7.0 4.5 10.5	2.5[b] 3.5	7.5	7.5	8.2	8.0	5.5	~ 4
$k_{20°} \times 10^4$ (sec^{-1})	0.01	0.01	0.015	0.015	—	—	0.2[a] 1.3	$\begin{bmatrix}1.7\\9.5\end{bmatrix}^{a}_{30°}$	0.6	$[1.8]_{30°}$	—	—
E (kcal/mol)	—	—	26.2	—	—	—	14[a] 26	—	18	—	—	—
% TR	85	65	100	90	>90	100[a] 80	90	85	100	90	>90	>80

[a] Reaction involves two steps.
[b] Quantum yield changes from upper to lower value as reaction progresses.

no longer forms a twin-stranded complex with poly-A. This difficulty has been overcome by treatment of the irradiated complex with ribonuclease, which hydrolyzes the poly-U chain and dissociates the double-stranded structure. It will be seen from Table I that Φ for uracil residues in the double-stranded complex is less than 25% of that in poly-U alone; but the percentage reversibility is much higher initially.

For poly-I – poly-C it is possible to follow the course of the photochemical reaction by direct spectral methods, taking advantage of the fact that the hyperchromicity of the complex is zero at 2700 Å, while poly-I alone is only slightly affected at this wavelength as a result of irradiation. The irradiated complex also exhibits the characteristic increase in absorption at 2400 Å due to formation of 5-hydro-6-hydroxycytosine derivatives[3,6-9]. The quantum yield is practically the same as for cytosine in poly-C; while the degree of reversibility, as estimated spectrally, is greater than 70%.

The foregoing results, particularly those obtained with the twin-stranded chains, reinforce previous evidence[1,3] that the reversible photolysis of pyrimidine residues in natural nucleic acids is a reasonable experimental model for biological photoreactivation and thermal reactivation.

The data in Table I also suggest some interesting conclusions with regard to energy transfer in polynucleotide chains, which has been assumed by some observers to play an important role in biological inactivation. For example, in a single-stranded chain such as poly-C, the %TR is high, indicating that photoproducts are almost exclusively 5-hydro-6-hydroxy derivatives. Poly-C is known to possess secondary structure; despite this the quantum yield for Cp residues in poly-C is independent of chain length and is also considerably lower than for free Cp[3]. It therefore follows that the photochemical reaction of individual Cp residues is unaffected by energy transfer along the chain, if such a transfer exists. Since the quantum yield for Cp in poly-I – poly-C is identical with that for poly-C, it likewise follows that energy transfer between chains, if it exists, also does not induce any photochemical reaction. A comparison of the quantum yields for uracil residues in poly-U and poly-A – poly-U leads to the same conclusion. A similar situation prevails for such dinucleotides as ApUp, ApCp, GpCp, the quantum yields for the pyrimidine residues in all instances being less than that for the free residues, notwithstanding that in a model dinucleotide such as DPN it has been shown that energy transfer actually does occur with fairly high efficiency[10].

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1 K. L. WIERZCHOWSKI AND D. SHUGAR, *Acta Biochim. Polon.*, 6 (1959) 313; 7 (1960) 377.
2 J. BARANOWSKA AND D. SHUGAR, *Acta Biochim. Polon.*, 7 (1960) 505.
3 D. SHUGAR AND K. L. WIERZCHOWSKI, *Postep Biochemii*, 4, Suppl. (1958) 243.
4 A. M. MOORE AND C. H. THOMSON, *Science*, 122 (1955) 594.
5 R. BEUKERS, J. IJLSTRA AND W. BERENDS, *Rec. trav. chim.*, 78 (1959) 883; 79 (1960) 101.
6 D. SHUGAR AND K. L. WIERZCHOWSKI, *Biochim. Biophys. Acta*, 23 (1957) 657.
7 K. L. WIERZCHOWSKI AND D. SHUGAR, *Biochim. Biophys. Acta*, 25 (1957) 355.
8 R. L. SINSHEIMER, *Radiation Research*, 1 (1954) 505.
9 C. JANION AND D. SHUGAR, *Acta Biochim. Polon.*, 7 (1960) 309.
10 G. WEBER, *J. chim. phys.*, 55 (1958) 878.

Zur Wirkung des UV-Lichtes auf die Proteinstruktur

Bekanntlich unterscheidet man in der Proteinchemie zwischen primärer, sekundärer und tertiärer Proteinstruktur. Durch die Sequenz der Aminosäuren in einer Peptidkette wird die primäre Struktur bestimmt. Das Ausmass, in dem sich die Peptidketten nach Pauling zu einer α-Helix aufwinden, charakterisiert die sekundäre Struktur. Die räumliche Orientierung dieser α Helix und auch der nicht gewendelten Peptidketten eines Proteinmoleküls nennt man dessen tertiäre Struktur.

Die wichtigste Bindungsart der primären Struktur ist die meist sehr feste kovalente C—C- oder C—N-Bindung (58.6 bzw. 48.6 kcal/Mol). Die α-Helix wird vor allem durch die recht labilen Protonenbrückenbindungen (5.0 kcal/Mol) zusammengehalten, während am Aufbau der tertiären Struktur ausser den erwähnten Protonenbindungen auch elektrostatische und van der Waals'sche Bindungskräfte, sowie in manchen Fällen auch kovalente S—S-Brücken (63.8 kcal/Mol) beteiligt sind.

Wird durch irgendeinen Prozess eine der erwähnten Strukturen verändert, so ist das untersuchte Protein nicht mehr nativ: es ist denaturiert, auch wenn es selbst in wesentlichen biologischen Eigenschaften noch mit dem Ausgangsprotein identisch erscheint.

Bei unseren Versuchen verwendeten wir als Bestrahlungsquelle einen Hg-Niederdruckbrenner (Hanau); Wellenlänge 254 mμ (297 mμ 0.8%; 302 mμ 0.3%; 313 mμ 3.0%); Quantenintensität in 4 cm Abstand: 0.4 μMol/cm^2 · min. Das UV-Licht der Wellenlänge 254 mμ ist mit einer Energie von mehr als 110 kcal/Mol Quanten bereits so energiereich, dass nach Absorption eines Quants jede der erwähnten Bindungen gesprengt werden kann. Darüberhinaus werden besonders die aromatischen Systeme des Tyrosins, Phenylalanins und Tryptophans zu sehr reaktionsfähigen Zuständen angeregt, welche bevorzugt mit dem Sauerstoffmolekül zu peroxydartig gebauten

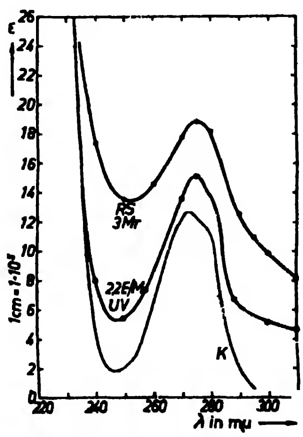

Abb. 1. Absorptionsspektren des Tyrosins: K = Kontrolle; mittlere Kurve: 0.1% Tyrosin in N/15 Phosphatpuffer pH 7.2, bestrahlt mit 2.2 Mol Quanten/Mol, λ = 254 mμ, obere Kurve = 0.1% Tyrosin in N/15 Phosphatpuffer bestrahlt mit 3 Mr Röntgenstrahlen. Luftgesättigte Lösungen. (Vergleich auch Lit.[1]).

Zwischenprodukten reagieren. Diese Intermediäreprodukte sind nicht zuletzt auch photochemisch sehr labil und zerfallen meist unter Lösung der O—O-Bindung. Durch ihren Zerfall wird eine Folge sehr komplizierter Reaktionen eingeleitet, deren letzte Stufe sehr oft ein Oxydationsprodukt des primär angeregten Aromaten ist.

Im Falle des Tyrosins und Phenylalanins kommt es zunächst zur Einführung neuer OH-Gruppen in den Benzolring. Dies führt besonders beim Tyrosin zu einer gleichmässigen Erhöhung der Extinktionskonstanten im Wellenbereich zwischen 220 und 310 mμ (Abb. 1). Im Falle des Phenylalanins scheint jedoch die oxydative Aufspaltung des aromatischen Ringes von Anfang an zu dominieren (keine Ausbildung eines Absorptionsmaximums bei 275 mμ). Weitere Oxydationsprozesse führen jedoch schliesslich auch beim Tyrosin zur Aufsprengung des Ringes und zur Bildung von zum Teil ungesättigten Ketosäuren oder zur Bildung von Melanin-Pigmenten (Abb. 2).

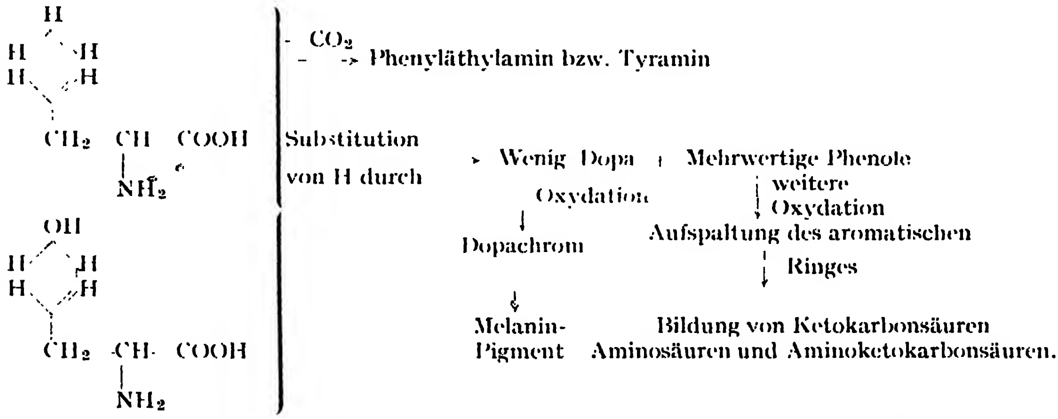

Abb. 2. Schema zum photochemischen Abbau des Phenylalanins und Tryptophans bei Bestrahlung in 0.1%iger, luftgesättigter Lösung (N/15 Phosphatpuffer pH 7.2). (Siehe auch Lit.[2].)

Wesentlich reaktionsfähiger ist der angeregte Indolring. Wie schon der Vergleich des UV-Absorptionsspektrums zwischen der Kontrolle und dem mit 2.2 E/Mol

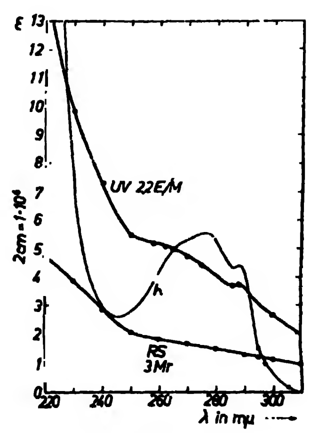

Abb. 3. UV-Absorptionsspektren des Tryptophans: K = Kontrolle; UV = 0.1% Tryptophan in $N/15$ Phosphatpuffer pH 7.2 bestrahlt mit 2.2 Mol Quanten/Mol, $\lambda = 254$ mμ; RS = 0.1 % Tryptophan bestrahlt mit 3 Mr Röntgenstrahlen. (Siehe auch Lit.[1].)

Literatur S. 617/618

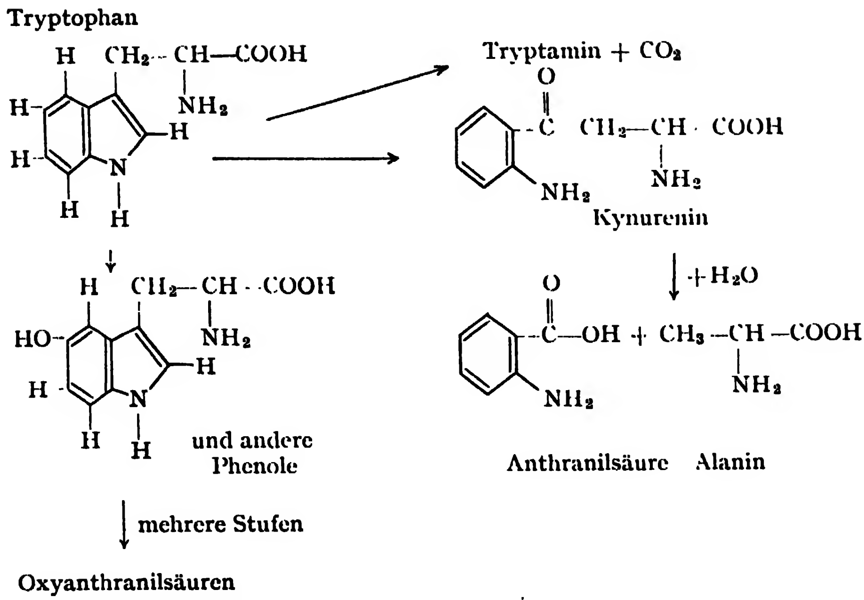

Abb. 4. Schema zum photochemischen Abbau des Tryptophans bei Bestrahlung in 0.1%iger luftgesättigter Lösung (N/15 Phosphatpuffer pH 7.2).

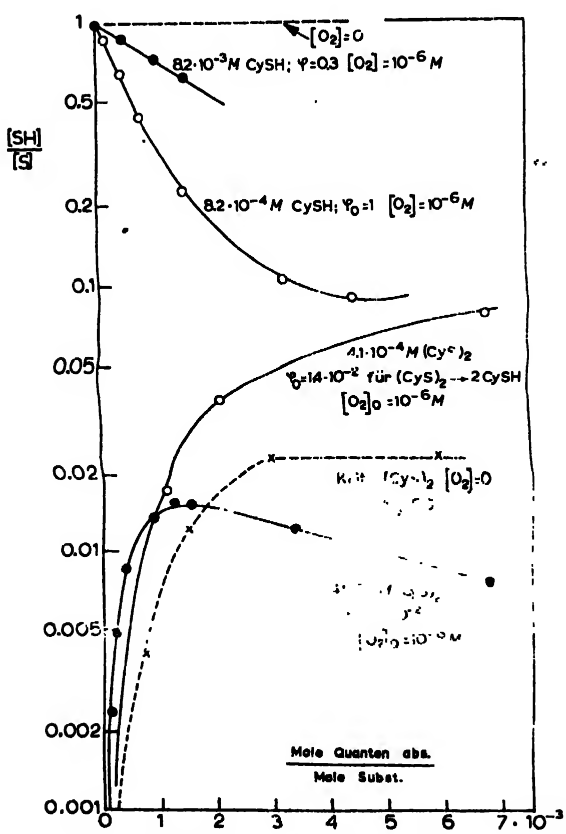

Abb. 5. Zum photochemischen Gleichgewicht bei Bestrahlung von Cystein (Cy SH) und Cystin $(Cy\text{-}S)_2$. Abszisse: Absorbierte Mole Quanten/Mol Substanz ($\lambda = 254$ mμ); Ordinate: [SH]/[S] (logarithmischer Massstab). Im Text diskutierte Kurven: --o - o--.

Quanten oder etwa 2 Mr bestrahlten Tryptophan zeigt, verschwindet der als chromophore Gruppe wirkende Pyrrol-Ring (Abb. 3). Die Oxydationsprozesse greifen besonders am Kohlenstoff-2 ein (Abb. 4). Unter den Abbauprodukten findet man das bekannte Kynurenin, sowie auch Anthranilsäure und Alanin. Parallel zu diesen Prozessen kommt es zu einer Einführung von OH-Gruppen in den Benzolring des Tryptophans. Bestrahlt man freies Tryptophan oder befindet sich diese Aminosäure an C-terminaler Stelle in einer Peptidkette, so wird leicht CO_2 abgespalten. So können bei gleichzeitiger Oxydation des Benzolringes die pharmakologisch stark wirksamen Oxytryptamine, wie z.B. das 5-Oxytryptamin entstehen.

Auch die Veränderung der schwefelhaltigen Aminosäuren hängt stark vom Sauerstoffgehalt des Mediums ab. Bei Bestrahlung SH-haltiger Verbindungen überwiegt natürlich im allgemeinen die photokatalysierte Oxydation zur S—S-Bindung. Aber auch die Umkehrung dieses Prozesses ist möglich[3], namentlich wenn die O_2-Partialdrucke klein oder die S—S-Bindung eines Proteins sehr gespannt ist. In Abb. 5 werden einige Ergebnisse über die Reversibilität des photochemischen Gleichge-

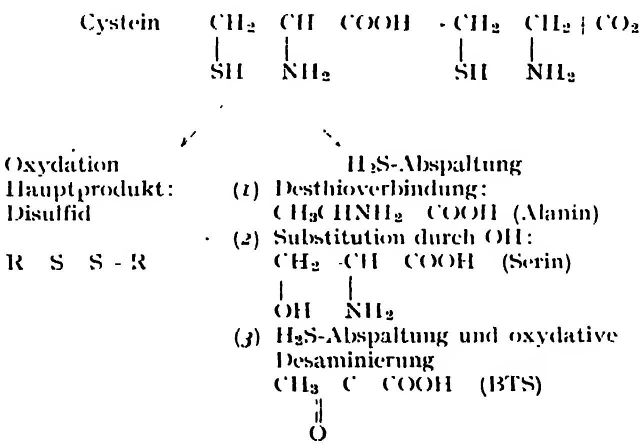

Abb. 6. Schema zur Photochemie des Cysteins.

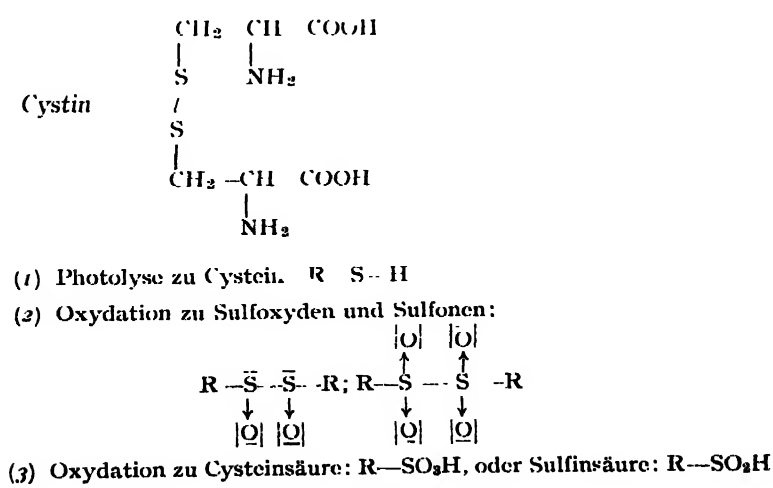

Abb. 7. Schema zur Photochemie des Cystins.

Literatur S. 617/618

wichts zwischen Cystein und Cystin graphisch dargestellt. Weitere Reaktionswege für die Photochemie der S-haltigen Aminosäuren Cystein, Cystin und Methionin zeigen die nächsten Abbildungen: (Abb. 6–8). Bemerkenswert ist die Entschwefelung zur Desthioverbindung: so entsteht Alanin aus Cystein oder α-Aminosäure aus Methionin; die Substitution der SH-Gruppe durch die OH-Gruppe führt zum Serin.

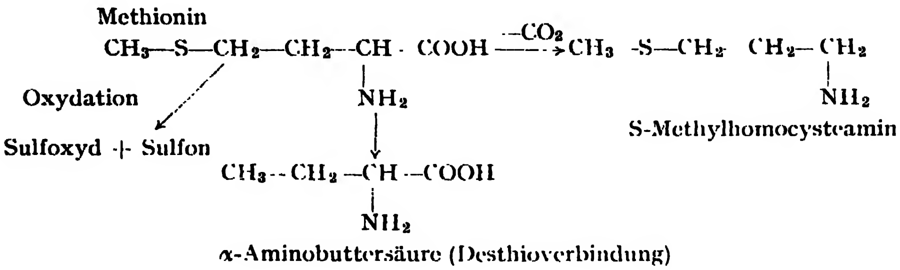

Abb. 8. Schema zur Photochemie des Methionins.

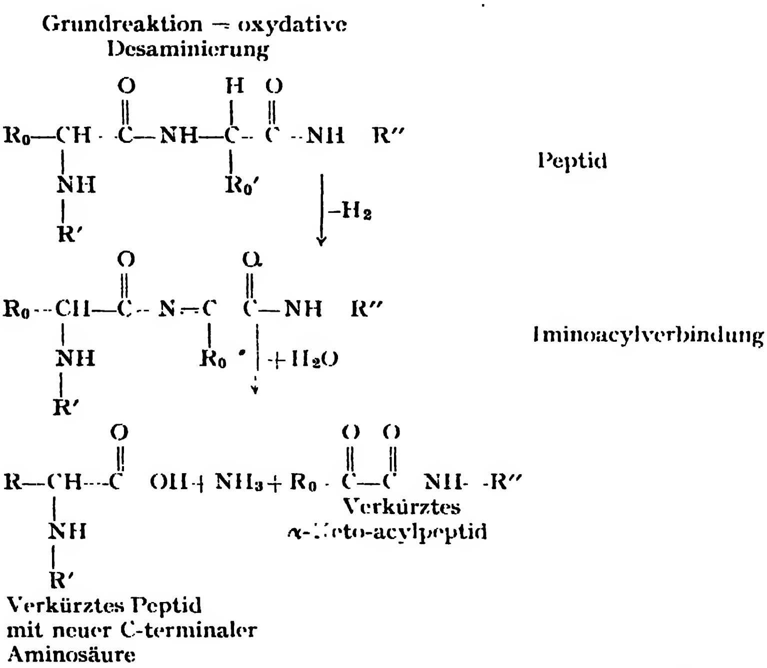

Abb. 9. Wichtige Teilreaktionen der strahlenchemischen Spaltung der Peptidbindung (Siehe auch Lit.[7, 8]).

Alle photochemischen Prozesse, welche die Aminoacyl-Reste der primären Struktur einfacherer Proteine betreffen, können grundsätzlich mit ähnlichen Quantenausbauten an den freien Aminosäuren selbst oder an deren niederen Peptiden gefunden werden. Wir haben diese Aussage besonders gründlich bei der photochemischen Untersuchung des Insulins und des Lysozyms prüfen können. (Alles Präparate von höchster Reinheit). Besonders die UV-Absorptionsspektren der tyrosinhaltigen Proteine erfahren bei 235 mμ und 275 mμ jedoch eine stärkere Extinktionserhöhung

als Aminosäuregemische gleicher Zusammensetzung, wenn man sie unter gleichen Bedingungen bestrahlt[4]. Bei Beteiligung der Peptidbindung an den photochemischen Prozessen beobachtet man eine interessante Reaktionsfolge, welche schliesslich zur Aufspaltung der Peptidbindung führt. Die wesentlichsten Schritte dieser Reaktion sind in Abb. 9 dargestellt.

Unsere Aussagen über die photochemische Veränderung der sekundären Struktur lassen sich wesentlich weniger leicht präzisieren. Da Harnstoff in vielen Fällen auch hier einer Koagulation entgegen wirkt, darf man jedoch vermuten, dass das Verhalten der Protonenbindungen auch bei der photochemisch ausgelösten Eiweisskoagulation von entscheidender Bedeutung ist.

Bezüglich der Veränderung der tertiären Struktur erwähnte ich bereits die teilweise reversible Lösung von S—S-Bindungen und die Schliessung neuer S—S-Bindungen. Auf Grund der fundamentalen Arbeiten des Kopenhagener Biochemikers K. Linderstrom-Lang[5] dürfen wir weiter schliessen, dass eine Veränderung der tertiären Struktur eines Proteins mit grosser Wahrscheinlichkeit zu einer Veränderung der Dissoziationskonstanten seiner polaren Gruppen führt. Kürzlich fanden wir nun z.B., dass reinste Lactatdehydrogenase (LDH) aus Schweineherzen (kristallisiert

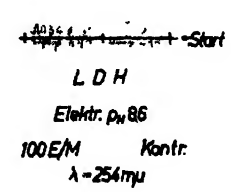

Abb. 10. Photochemische Bildung einer neuen Lactatdehydrogenase (LDH) durch Bestrahlung von LDH aus Schweineherz. Niederspannungspapier-Elektrophorese. Absorbierte Quanten: 100 Mol Quanten/Mol. Quantenausbeute $\varphi = 1.27 \cdot 10^{-2}$.

nach Straub[6]) bei Bestrahlung in Lösung mit der Quantenausbeute von $\varphi = 0.5 \cdot 10^{-2}$ fast quantitativ in eine bei pH 8.6 im elektrischen Feld langsamer wandernde LDH umgewandelt wird. Dieses neue Enzym hat noch etwa 50% der Aktivität des ursprünglichen Enzyms (Abb. 10); jedoch ist das 20°C-pH-Optimum von pH 8.1 nach pH 7 verschoben. Unsere bisherigen Ergebnisse sprechen dafür, dass für die Veränderung der elektrochemischen Eigenschaften dieses neuen Proteins nicht eine photochemische Veränderung der primären, sondern der sekundären und tertiären Struktur verantwortlich ist.

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1 I. MORING-CLAESSON, *Arkiv Kemi*, 10 (1956) 1.
2 P. GONNARD, *Am. pharm. franç.*, 11 (1953) 283.

[3] K. SCHOCHEN, *Science*, 116 (1952) 544.
[4] D. J. McLEAN UND A. C. GIESE, *J. Biol. Chem.*, 187 (1950) 537.
[5] K. LINDERSTRØM-LANG UND S. O. NIELSEN, *Acid Base Equilibria of Proteins*, in M. BIER (ed.), *Electrophoresis*, Academic Press, New York, 1959.
[6] F. STRAUB, *Biochem. J.*, 34 (1940) 483.
[7] A. D. McLAREN, P. GENTILE, D. C. KIRCK JR. UND N. A. LEVIN, *J. Polymer Sci.*, 10 (1953) 333.
[8] D. C. CARPENTER, *J. Franklin Inst.*, 232 (1941) 76.
[9] B. RAJEWSKY UND K. DOSE, *Z. Naturforsch.*, 12b (1957) 384.

Über den photosensibilisierten Abbau von Makromolekülen

1954 berichtete P. Alexander[1] über den photodynamischen Abbau von Makromolekülen nach Sensibilisierung durch Farbstoffe und cancerogene Kohlenwasserstoffe. Danach wird in wässrigen Lösungen der photodynamische Effekt durch HO_2-Radikale verursacht, die durch Reaktion des gelösten Sauerstoffs mit dem aktivierten Sensibilisator gebildet werden. Als Makromolekül wurde unter anderem die Polymethacrylsäure verwendet.

Von Interesse schien uns, ob auch in nichtwässrigen Lösungssystemen Makromoleküle durch den cancerogenen Kohlenwasserstoff 3,4-Benzpyren bei UV-Einwirkung abgebaut werden können. Für diese Untersuchungen wurden Polymethacrylester sowie die Lösungsmittel Benzol, Chloroform und Dioxan verwendet. In diesen Lösungsmitteln wird das Polymer durch UV-Strahlung unterhalb 295 mμ sehr rasch abgebaut. Durch Strahlung im längerwelligen UV-Gebiet dagegen nur langsam[2].

Die Konzentration des Polymers betrug in allen Lösungsmitteln 0.4 g/l, die Benzpyren-Konzentration in Benzol und Dioxan je 20 mg/l und in Chloroform 40 mg/l. Bestrahlt wurde mit einem Quarzquecksilber-Hochdruckbrenner vom Typ PRK 2 (S 450) des VEB Berliner Glühlampenwerk bei Zimmertemperatur. Bei Verwendung eines Wasserfilters von 20 mm Schichtdicke in einer Küvette, die beiderseits mit je 2 mm dicken WG 5 - Filtergläsern der Firma Schott, Jena, abgedeckt war, betrug in einem Abstand von 9 cm die Strahlungsintensität $2.35 \cdot 10^5$ erg/cm^2 sec. Die

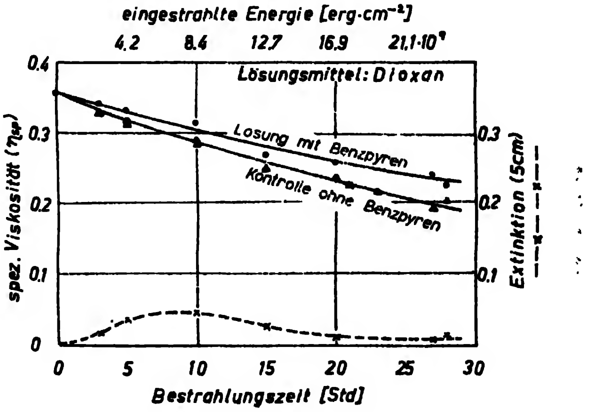

Abb. 1. Der Verlauf der Extinktion und spez. Viskosität in Abhängigkeit von der Bestrahlungszeit nach UV-Einwirkung auf Polymethacrylester–Benzpyren-Lösungen in Dioxan. - - - - - - - -: Extinktion; ●——●——●: spez. Viskosität; △——△——△: spez. Viskosität der Kontrolllösung (Polymer in Dioxan ohne Benzpyren).

Filtergläser absorbieren das UV-Licht unterhalb 295 mμ. Die Bestrahlung der Lösungen erfolgte in einer Quarzküvette von 10 mm Schichtdicke, die geeignet war, die Lösungen vor der Bestrahlung mit O_2 bzw. N_2 zu durchströmen. Der Abbau der Makromoleküle wurde viskosimetrisch bestimmt, wobei als Mass die spezifische Viskosität (η_{sp}) Verwendung fand. Die bei der Bestrahlung der Lösungen auftretenden Gelbfärbungen konnten mit dem Pulfrich-Photometer unter Verwendung des Filters S 50 (wirksamer Filterschwerpunkt bei 496 mμ) gemessen werden (Bestimmung der Extinktion). Die zur Messung der Extinktionen verwendeten Schichtdicken lagen zwischen 1 und 5 cm.

Verwendet man als Lösungsmittel Dioxan, so erhält man über mehrere Stunden Bestrahlungszeit einen Abbau des Polymers, auch ohne Zusatz eines Sensibilisators (s. Abb. 1). Fügt man der Lösung 3,4-Benzpyren hinzu, so ist der Abbau vermindert. Diese Verminderung ist offensichtlich auf die Filterwirkung des Benzpyrens zurückzuführen. Bei der Dioxanlösung liegt also kein Sensibilisierungseffekt vor. Wie weiter aus Abb. 1 zu ersehen ist, tritt bei Bestrahlung eine Verfärbung und zwar eine Gelbfärbung der Lösung auf (gestrichelte Kurve), die bei der Lösung ohne Benzpyren nicht vorkommt. Die Verfärbung steigt zunächst mit zunehmender Bestrahlungszeit an, um nach Erreichen eines Maximums wieder abzunehmen. Die Extinktion dieser Gelbfärbung ist sehr gering. Sie wurde mit Schichtdicken von 5 cm bestimmt. Mit zu-

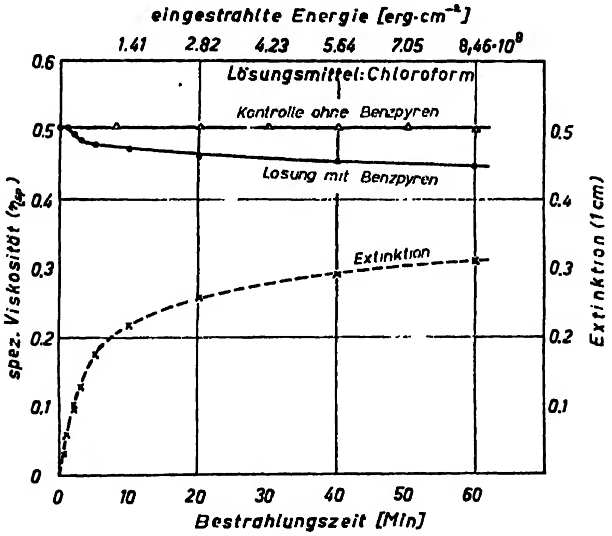

Abb. 2. Der Verlauf von Extinktion und spez. Viskosität in Abhängigkeit von der Bestrahlungszeit nach UV-Einwirkung auf Polymethacrylester–Benzpyren-Lösungen in Chloroform. – – – – –: Extinktion; ●-- ●— -●: spez. Viskosität; △- - △ - - △: spez. Viskosität der Kontrolllösung (Polymer in Chloroform ohne Benzpyren).

nehmender Extinktion der Lösung verringert sich gleichzeitig die charakteristische Benzpyren-Fluoreszenz, die jedoch mit abnehmender Extinktion nicht wieder auftritt.

Verwendet man als Lösungsmittel Chloroform (für diese Versuche wurde handelsübliches Chloroform mit *ca.* 1% Alkohol verwendet), so erhält man nach einer kurzen Induktionsperiode einen geringfügigen Abbau (s. Abb. 2). Auch hier tritt eine Gelbfärbung der Lösung auf, die allerdings gegenüber der Dioxanlösung sehr stark ist. Die

Literatur S. 621

Extinktion dieser Verfärbung wurde bei 1 cm Schichtdicke gemessen. Der Extinktionsverlauf (gestrichelte Kurve) entspricht der Fluoreszenzabnahme des Benzpyrens in Chloroform[3]. Bei mehrstündiger Bestrahlung tritt ein allmähliches Ausbleichen des Farbstoffs auf. Versuche, bei denen vor der Bestrahlung der Sauerstoff durch Stickstoffspülung entfernt wurde, zeigen, dass der Abbau auch ohne Sauerstoff vor sich geht. Ebenso tritt eine Gelbfärbung auf, die jedoch vermindert ist. Zur Deutung des Abbaus könnte man daran denken einen Chlorierungsmechanismus anzunehmen, ähnlich, wie er von verschiedenen Autoren[4] bei der Photochlorierung des Polymethacrylesters gefunden worden ist. Danach wird durch freie Chlorradikale dem Polymer ein Wasserstoffatom entzogen, worauf durch Disproportionierung ein Hauptkettenbruch eintritt.

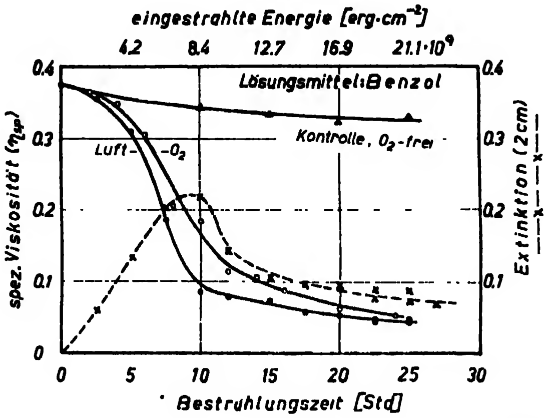

Abb. 3. Der Verlauf von Extinktion und spez. Viskosität in Abhängigkeit von der Bestrahlungszeit nach UV-Einwirkung auf Polymethacrylester Benzpyren-Lösungen in Benzol unter Berücksichtigung der O_2-Abhängigkeit. • : Extinktion (O_2-Spannung unter Luftatmosphäre); ● ● ---●: spez. Viskosität (O_2-Spannung unter Luftatmosphäre); ○ ○ ○: spez. Viskosität (Lösung mit O_2 durchströmt); ▲ ▲ ▲: spez. Viskosität (Lösung O_2-frei).

Verwendet man als Lösungsmittel Benzol, so tritt bei Anwesenheit von Benzpyren ebenfalls eine Gelbfärbung auf, deren Extinktionsverlauf (gestrichelte Kurve) qualitativ dem Extinktionsverlauf bei der Dioxanlösung entspricht (s. Abb. 3). Allerdings ist hier die Färbung erheblich stärker. Die Extinktion wurde mit Schichtdicken von 2 cm gemessen. Mit zunehmender Extinktion verringert sich gleichzeitig auch hier die charakteristische Benzpyren-Fluoreszenz[3], die mit abnehmender Extinktion nicht wieder auftritt. Mit zunehmender Verfärbung der Lösung wird nach einer kurzen Anlaufperiode eine starke Abnahme der spezifischen Viskosität beobachtet. Die Abbaugeschwindigkeit wird erst geringer, nachdem die Extinktionswerte ihr Maximum überschritten haben. Entfernt man nämlich vor der Bestrahlung den Sauerstoff durch Spülung mit Stickstoff, so findet ein geringfügiger Abbau statt, der jedoch noch kleiner ist als bei einer Kontrollösung, die ohne Benzpyren bestrahlt worden ist (die Werte für die Kontrollösung ohne Benzpyren sind in Abb. 3 nicht eingetragen). In der Lösung ohne O_2 tritt auch keine Gelbfärbung auf.

Durchströmt man vor der Bestrahlung die benzolische Benzpyren-Lösung bis zur

Sättigung mit O_2, so wird die Abbaugeschwindigkeit gegenüber den Versuchen bei Luftatmosphäre nicht erhöht, sondern im Gegenteil vermindert (s. O_2-Kurve in Abb. 3). Der Extinktionsverlauf bei erhöhter O_2-Spannung entspricht qualitativ dem Verlauf bei Luftatmosphäre (in Abb. 3 nicht eingezeichnet). Aus diesem Versuch erkennen wir, dass der Sauerstoff auch eine inhibierende Wirkung auf den Abbau des Polymethacrylesters ausübt und dass es ein Optimum an gelöster O_2-Menge geben muss, bei dem die Abbaugeschwindigkeit am grössten ist.

Zur Deutung der für die benzolische Lösung mitgeteilten Ergebnisse muss man bemerken, dass bei Bestrahlung des Polymethacrylesters in Benzol ohne Zusatz von Benzpyren mit kurzwelligem UV-Licht unter anderem auch ein oxydativer Abbau stattfindet. Die hier beschriebenen Versuche lassen sich möglicherweise durch den von Schönberg[5] sowie von Schenck[6] formulierten Mechanismus der Sauerstoffübertragung deuten, indem auch dieser Abbau durch oxydative Vorgänge ausgelöst wird. Diese Vermutung wird bestärkt, da Untersuchungen von Schenck[7] gezeigt haben, dass das 3,4-Benzpyren ein vorzüglicher Photosensibilisator ist. Da von den drei untersuchten Lösungsmitteln eine Sensibilisatorwirkung unter Beteiligung des Sauerstoffs nur beim Benzol vorliegt, kann man schliessen, dass ein Akzeptor für den Sauerstoff nur in benzolischer Lösung vorliegt oder entsprechend der längeren Anlaufzeit für den Abbau erst unter Bestrahlung gebildet wird. Auf den letzteren Vorgang deutet auch der Zusammenhang zwischen zunehmender Extinktion und abnehmender spezifischer Viskosität hin. Nach diesen Ergebnissen lässt sich allerdings nicht ausschliessen, dass möglicherweise erst das unter Bestrahlung veränderte Benzpyrenmolekül als Sauerstoffüberträger dient. Weitere Untersuchungen zu diesen Vorgängen sollen durchgeführt werden.

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[1] P. Alexander und M. Fox, *Proc. I. Intern. Photobio. Congr., Amsterdam 1954*, Veenman, Wageningen, p. 336.
[2] H. Mönig, *Probleme aus Biophysik und Strahlenbiologie*, Bd. 2, Berlin, 1960, p. 174.
[3] H. Kriegel und L. Herforth, *Z. Naturforsch.*, 12b (1957) 41.
[4] W. Hahn und F. Grafmüller, *Makromol. Chem.*, 21 (1956) 121.
G. Smets und J. Fournaux, *Makromol. Chem.*, 24 (1957) 133.
[5] A. Schönberg, *Präparative organische Photochemie*, Springer, Berlin, 1958.
[6] G. O. Schenck, s.z.B. *Angew. Chem.*, 69 (1957) 579.
[7] G. O. Schenck, *Naturwissenschaften*, 43 (1956) 71.

AUTHOR INDEX

SUBJECT INDEX